32nd ANNUAL GRADUATE RESEARCH SYMPOSIUM

VIRGINIA-MARYLAND COLLEGE OF VETERINARY MEDICINE

March 14, 2023 • Blacksburg, VA • 7:30 AM to 7:30 PM

Welcome to the 32nd Annual Graduate Research Symposium

Each year, the Virginia-Maryland College of Veterinary Medicine hosts this event to support the College's mission of providing education to a diverse population of professional and post-graduate students in preparation for careers in the broad areas of veterinary medicine, biomedical sciences, and public health. The event serves to showcase the research of our graduate and training programs. The MS and Ph.D. degree curriculum operates as a single multi-disciplinary Biomedical and Veterinary Sciences (BMVS) graduate program.

For more information about our program, please visit our new program website:
bmvs.vetmed.vt.edu

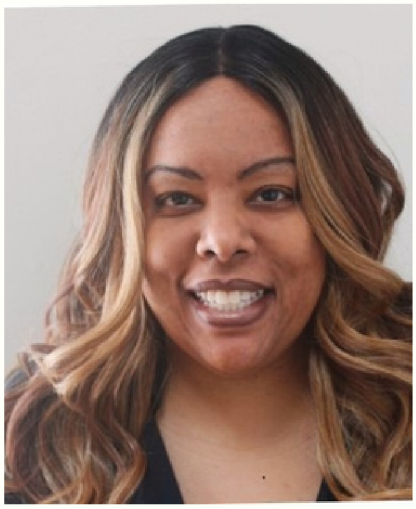
BIOMEDICAL AND VETERINARY SCIENCES
VIRGINIA TECH™

PROGRAM AT A GLANCE

Social Determinants of Animal and Human Health

7:30 am	Registration and Continental Breakfast
8:15 am	Poster Session I
9:45 am	Welcome and Introduction
10:00 am	**Keynote Speaker Dr. LeeAnn Bailey**
11:00 am	Oral Session I
12:00 pm	Lunch for Registered Participants
1:00 pm	**Keynote Speaker Dr. Melinda Beck**
2:00 pm	Poster Session II
3:30 pm	Oral Session II
4:30 pm	Flash Talks
5:00 pm	Social Hour for Registered Participants
6:00 pm	Dinner for Registered Participants
6:30 pm	Awards Ceremony for Registered Participants

Keynote Speakers & Oral Presentations: Classroom 100
Poster Presentations & Food Service: CVM Student Lounge [Waterfall Room]
Social Hour & Dinner: University Club



Dr. LeeAnn Bailey

Branch Chief
Integrated Networks Branch at National Cancer Institute (NCI)
Center to Reduce Cancer Health Disparities

LeeAnn Bailey is Chief of the Integrated Networks Branch of the NCI's CRCHD. In this role, she manages, develops, and assesses strategies for enhancing the integration and dissemination of diversity training, women's health, and sexual and gender minority efforts within and across NCI, as well as within the scientific community and underserved communities through NCI-supported networks. She also identifies and leverages opportunities to address unmet needs in cancer health disparities research.

Prior to joining NCI, she was a healthcare consultant at Deloitte Consulting LLP. She has also been a principal investigator researching tissue engineered products and cellular inflammatory responses at the National Institute of Standards and Technology as well as an adjunct professor at Morgan State University.

Dr. Bailey received her M.B.B.S (M.D. equivalent) from the University of Adelaide Medical School with an emphasis on aboriginal health and pediatric oncology. Dr. Bailey also has a Ph.D. in Biochemistry and Molecular Genetics and a M.S. in Biological and Physical Sciences from the University of Virginia School of Medicine.

Keynote Speaker 10 am
Classroom 100 and Zoom

Dr. Melinda Beck

Interim Chair and Professor Department of Nutrition
Gillings School of Global Public Health
University of North Carolina

Melinda Beck, PhD, serves as interim chair of the Department of Nutrition at the Gillings School of Global Public Health. Dr. Beck has been a faculty member at UNC since 1992 and joined the Department of Nutrition faculty in 1996. Currently, she is a professor of nutrition and the associate chair for academics within the department. Beck earned a bachelor's degree in zoology from the University of California; a Master of Science in biological sciences from California Polytechnic University; and a doctorate in microbiology/immunology from The Ohio State University.

In Beck's laboratory, researchers study the relationship between host nutrition and the immune response to infectious disease, including an ongoing clinical study of the mechanisms that impair influenza vaccine response in obese adults compared with healthy weight adults. This approach has evolved over the years as she has come to appreciate the complex ways in which an individual's nutritional status can affect the course and severity of virally induced diseases. Dr. Beck's earliest work examined the molecular basis for the effect of selenium deficiency on the severity of coxsackievirus B3-induced cardiomyopathy. This work led to the understanding that nutritional deficiencies impact viral evolution. Thus, host nutritional status should be considered as a driving force for viral evolution and emerging infectious diseases.

More recently, Dr. Beck's work has expanded to focus on the intersection between a non-communicable disease, obesity, and a communicable disease, influenza. Her research group was among the first to demonstrate that diet-induced obese mice are highly susceptible to increased morbidity and mortality from infection with influenza. This has also been found to be true for obese human populations as well. Recently, obesity is also an identified risk factor for severe outcome from SARS-CoV-2 infections. Dr. Beck's current research is focused on understanding the mechanism(s) involved in obesity-induced impaired immunity to infectious disease.

Keynote Speaker 1 pm
Classroom 100

DETAILED SCHEDULE

7:30 am **Registration and Continental Breakfast**
CVM Student Lounge [Waterfall Room]

8:15 am **Poster Session I**
CVM Student Lounge [Waterfall Room]

9:45 am **Welcome and Introduction**
Classroom 100

Dr. Ansar Ahmed
Associate Dean for Research and Graduate Studies

10:00 am **Keynote Speaker Dr. LeeAnn Bailey**
"Addressing Social Determinants of Health Through Research and Training"
Classroom 100 and Zoom
Moderator: Kelsey Murphy

11:00 am **Oral Session I: Student Presentations**
Chair: Mitch Caudill
Classroom 100

"Outcomes of hyperthyroid cats treated with radioiodine (I-131) using a variable-dose protocol determined by serum thyroxine (T4) concentration"
Michael Ciepluch

"Teaching an old drug a new trick: Repurposing the anti-inflammatory FDA-approved drug, auranofin, to treat *Neisseria gonorrhoeae*"
Hsin-Wen Liang

SCHEDULE, CONT

11:00 am, CONT	**"The Role of Sympathetic Neuronal Pathways in Regulating HSV1 and HSV2 Genital Infection"** Greyson Moore
	"High-frequency irreversible electroporation of glioma alters tumor-derived extracellular vesicles to mediate blood-brain barrier disruption" Kelsey Murphy
	"Evaluation of neurofilament light chain as a biomarker in dogs with structural and idiopathic epilepsy" Kayla Fowler
12:00 pm	**Lunch for Registered Participants** *CVM Student Lounge [Waterfall Room]*
1:00 pm	**Keynote Speaker Dr. Melinda Beck** **"Obesity and Influenza: A Public Health Crisis"** *Classroom 100* Moderator: Jus Tupik
2:00 pm	**Poster Session II** *CVM Student Lounge [Waterfall Room]*
3:30 pm	**Oral Session II: Student Presentations** *Chair: Lauren Panny* *Classroom 100*
	"A novel 3D printed instrument enhances removal of equine guttural pouch chondroid prostheses" Guillermo Cardona

SCHEDULE, CONT

3:30 pm, CONT

"A hepatitis B core antigen-based virus-like particle vaccine expressing SARS-CoV-2 T and B cell epitopes induces epitope-specific humoral and a cell-mediated immune responses"
Anna Hassebroek

"Equine bone marrow-derived mesenchymal stromal cells disrupt the matrix of established orthopedic biofilms in vitro"
Sarah Khatibzabeh

"Human rotavirus replicates in the salivary glands and induces IgM responses in the facial lymphoid tissues of gnotobiotic pigs"
Charlotte Nyblade

"High Frequency Irreversible Electroporation (H-FIRE) of canine hepatocellular carcinoma results in immediate quantitative and qualitative changes in peripheral blood circulating extracellular vesicles"
Alejandra Tellez Silva

4:30 pm

Flash Talks: Student Presentations
Chair: Dr. Sarah Khatibzadeh
Classroom 100

"A potent antibacterial activity against *Clostridioides difficile* is exhibited by antifungal drugs"
Ahmed Abouelkhair

SCHEDULE, CONT

4:30 pm, CONT

"Construction of novel antibiotic-resistant mutants of *Neisseria gonorrhoeae* strains suitable for mouse vaginal infection model"
Babatomiwa Kikiowo

"Discovering Antiviral Treatments for Alphaviruses Through Proteomic Analysis of the VEEV E1 Glycoprotein"
Lauren Panny

"Evaluation of a feline optimized TSH assay in cats with hyperthyroidism and with non-thyroidal illness"
Camille Brassard

"Combined live oral priming and intramuscular boosting regimen with Rotarix and a nanoparticle-based multivalent rotavirus vaccine confers significant protection against both G4P[6] and G1P[8] human rotavirus infection in gnotobiotic pigs"
Casey Hensley

"Exploiting virus-host interactions to develop novel inhibitors against Venezuelan equine encephalitis virus"
Abdullahi Jamiu

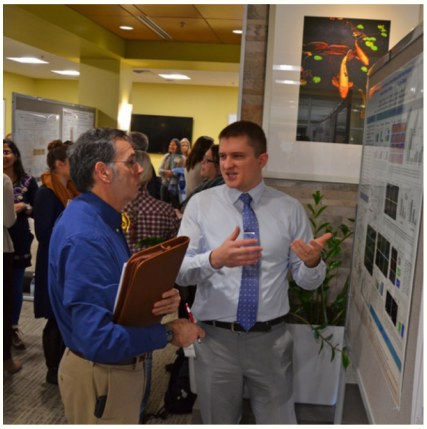
"Time is Brain: Stimulating Tie2 Improves Pial Collateral Growth and Reduces Tissue Damage After Ischemic Stroke"
Alexandra Kaloss

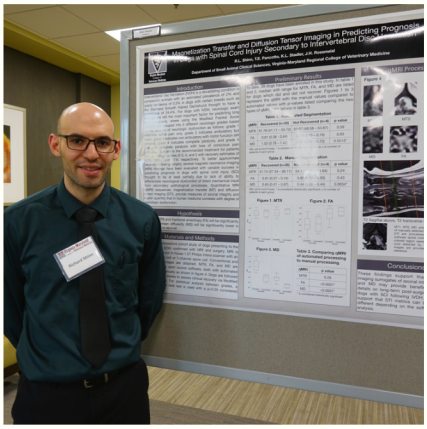
SCHEDULE, CONT

5:00 pm **Social Hour for Registered Participants**
University Club

6:00 pm **Banquet Dinner for Registered Participants**
University Club

6:30 pm **Awards Ceremony for Registered Participants**
University Club





POSTER PRESENTERS

Session 1

Block 1 8:15 - 9:00 am

2. Allie Kaloss
4. Ester Yang
6. Jatia Mills
8. Josefa Garcia
18. Rafaela Machado Flor
20. Xiaoran Wei
22. Christina Chuong
24. Michael Brooks
34. Ahmed Abouelkhair
36. Babatomiwa Kikiowo
38. Lauren Panny
40. Steven Hanes

Block 2 9:00 - 9:45 am

10. Michael Edwards
12. Morgen VanderGiessen
14. Kaylee Petraccione
16. Swagatika Paul
26. Jus Tupik
28. Mitch Caudill
30. Brie Trusiano
32. Nadia Saklou
42. Camille Brassard
44. Casey Hensley
46. Abdullahi Jamiu
47. Peixi Chang
48. Nicole Sugai
50. Anna Zimina
52. Urmil Dave

POSTER PRESENTERS

Session 2

Block 1 2:00 - 2:45 pm

1. Samantha Barth
3. Tosin Ogunmaupwa
5. Amir Mortazavigazar
7. Holly Morrison
17. Cody Swilley
19. Jing Ju
21. Tristan Stoyanof
23. Maria Ruiz Diaz
33. Lauren Helber
35. Ehab Salama
37. Yehia Engammal
39. Catherine Jula

Block 2 2:45 - 3:30 pm

9. Brittany Heath
11. Razan Alajoleen
13. Caitlin Armstrong
15. Dao Xu
25. Nicolas Burns
27. Pallavi Rai
29. Christina Pacholec
31. Nour Aklashef
41. Dima Hajj Ali
43. Padmaja Mandadi
45. Laura Bruckner
51. Xiguang Xu
53. Yu Lin
54. Mahshid Arabi

ACKNOWLEDGMENTS

After a challenging past two years due to the pandemic, we are delighted to host our 32nd Annual Graduate Research Symposium event in-person. This year's theme is "Social Determinants of Animal and Human Health." On behalf of the College of Veterinary Medicine, I want to thank our invited speakers, Dr. Leann Bailey from the National Cancer Institute/NIH and Dr. Melinda Beck from Gillings School of Global Health, the University of North Carolina, for agreeing to deliver the keynote talks.

Our Research Symposium is graduate student-centric and is designed to celebrate and showcase VMCVM graduate students' ongoing research projects. This event is a collaboration of the Office of Research and Graduate Studies with the Biomedical and Veterinary Sciences (BMVS) Graduate Students Executive Committee. I sincerely thank personnel from the Office of Research and Graduate Studies, Dr. Jessica Crawford, Andrea Green, and Monica Taylor, and I want to thank our Graduate Teaching Assistants Caitlin Armstrong and Brittany Heath, as well as the members of the Graduate Students Executive Committee members: Kelsey Murphy, Jus Tupik, Allie Kaloss, Tristan Stoyanof, Mitch Caudill, Alessandra Franshini, Brie Trusiano, Charlotte Nyblade, Amir Mortazavigazar, Lauren Panny, Sarah Khatibzadeh, and Jatia Mills who assisted in the symposium. I also take this opportunity to the members of the faculty of the VMCVM Research Committee for reviewing abstracts and the faculty for serving as judges to evaluate presentations.

We sincerely appreciate the support of the sponsors Zoetis, William A. Truban VMD Visiting Scholar Endowment Fund, and the College for supporting this symposium.

--Dr. Ansar Ahmed, Associate Dean of Research and Graduate Studies

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SESSION SCHEDULE

POSTER SESSION #1: BLOCK 1
8:15 AM - 9:00 AM
WATERFALL ROOM

P2 Alexandra M. Kaloss, Kennedie Lyles, Nathalie A. Groot, Jackie Zhu, John B. Matson, and Michelle H. Theus

Time is Brain: Stimulating Tie2 Improves Pial Collateral Growth and Reduces Tissue Damage After Ischemic Stroke

P4 Ester Yang, Lauren Ruger, Jessica Gannon, Hannah Sheppard, Sheryl Coutermarsh-Ott, Timothy J. Ziemlewicz, Nikolaos Dervisis, Irving C. Allen, Gregory B. Daniel, Joanne Tuohy, Eli Vlaisavljevich, Shawna Klahn

Mechanical High-Intensity Focused Ultrasound (Histotripsy) in Dogs with Spontaneously Occurring Soft Tissue Sarcomas

P6 Jatia Mills, Eman Soliman, Jing Ju, Alexandra M. Kaloss, Erwin Kristobal Gudenschwager Basso, Nathalie Groot, Colin Kelly, Elizabeth A. Kowalski, Mohamed Elhassanny, Michael Chen, Xia Wang, and Michelle H. Theus

Non-essential role for Cx3cr1-expressing EPH receptor A4 in a murine model of TBI

P8 Josefa Garcia-Mora, Rell L. Parker, Thomas Cecere, John L. Robertson, John H. Rossmeisl

The T2-FLAIR mismatch sign as an imaging biomarker for canine oligodendrogliomas

P18 Rafaela Flor, Amy Smith, Alan Baer, Kimberley Alex Hodge, Emanuel F. Petricoin, Jon D. Dinman, Jonathan Jacobs, Kehn-Hall

Transcriptomic and Proteomic Analysis of Venezuelan and Eastern Equine Encephalitis Virus Infected Astrocytes

P20 Xiaoran Wei, Leanne M Holt, Natasha L Pacheco, Boyu Lyu, Raymundo Hernandez, Guoqiang Yu and Michelle L Olsen

Transcriptomic Profiling of Astrocyte Development

P22 Christina Chuong, Chelsea Cereghino, James Weger-Lucarelli

Attenuation mechanism of an interferon gamma expressing chikungunya vaccine

P24 Michael Brooks, Yuchin Albert Pan

Regulation of developmental cell death in hypothalamic corticotrophin-releasing hormone neurons through DSCAML1 and cortisol signaling

P34 Ahmed A. Abouelkhair, Nader S. Abutaleb, Mohamed N. Seleem

A potent antibacterial activity against Clostridioides difficile is exhibited by antifungal drugs

P36 Babatomiwa Kikiowo, Aloka B. Bandara, Nader S. Abutaleb, and Mohamed N. Seleem

Construction of novel antibiotic-resistant mutants of Neisseria gonorrhoeae strains suitable for mouse vaginal infection model

P38 Lauren Panny, Ivan Akrhymuk, Nicole Bracci, Caitlin Woodson, Rafaela Flor, Weidong Zhou, Aarthi Narayanan, Catherine Campbell, Kylene Kehn-Hall

Discovering Antiviral Treatments for Alphaviruses Through Proteomic Analysis of the VEEV E1 Glycoprotein

P40 SR Hanes, RV Ramos, IP Herring

Pre- and Post-Phacoemulsification Morphologic Iridocorneal Angle Analysis by Anterior Segment Gonioscopic Imaging as a Predictor of Glaucoma in Dogs

SESSION SCHEDULE

POSTER SESSION #1: BLOCK 2
9:00 AM - 9:45 AM
WATERFALL ROOM

P10 Michael Edwards, John Rossmeisl, Gregory Daniel, Richard Shinn
Comparison of semi-automated and automated diffusion tensor imaging scalar value acquisition in dogs

P12 Morgen VanderGiessen, Victoria Callahan, Brian Carey, Kylene Kehn-Hall
HDM2 inhibitor NVP-CGM097 significantly reduces alphavirus replication in microglia

P14 Kaylee Petraccione, Mohamed Ali, Nicole Bracci, Normand Cyr, Haytham M. Wahba, Andrew Silberfarb, Danuta Sastre, Paul O'Maille, James Omichinski, Kylene Kehn-Hall
Rift Valley fever virus NSs protein interacts with LC3 family members to modulate autophagy

P16 Swagatika Paul, Shireen A. Sarraf, Ki Hong Nam, Leila Zavar, Sahitya Ranjan Biswas, Lauren E. Fritsch, Nicole DeFoor, Tomer M. Yaron, Jared L. Johnson, Emily M. Huntsman, Lewis C. Cantley, Alban Ordureau, and Alicia M. Pickrell
NAK associated protein 1/NAP1 is required for mitosis and cytokinesis through TBK1 activation

P26 Juselyn D. Tupik; Mecaila E. McClune; Hailey W. Camp; Julia A. Gregory; Jules M. Dressler; Justin W. Markov Madanick; Margaret A. Nagai-Singer; Brandon L. Jutras; Irving C. Allen
Mounting a mitochondrial defense: Innate immune receptor NLRX1 regulates cell metabolism and death for protection against Lyme disease

P28 Mitchell T. Caudill and Clayton C. Caswell
Regulation of and by Quorum Sensing Proteins in the Zoonotic Pathogen Brucella abortus

P30 Brie Trusiano, I.C. Allen
Proliferation, Plasticity, and Migration: Exploring the synergistic relationship between eosinophils and TH2 cells in development of HES-like syndrome

P32 Nadia Saklou, Brandy Burgess, Kevin Lahmers
LAMPore sequencing as a novel diagnostic for equine herpesvirus 1 using equine nasal swabs

P42 Camille Brassard, Stephanie DeMonaco
Evaluation of a feline optimized TSH assay in cats with hyperthyroidism and with non-thyroidal illness

P44 Casey Hensley, Charlotte Nyblade, Peng Zhou, Viviana Parreno, Annie Frazier, Maggie Frazier, Ariana Fantasia-Davis, Sarah Garrison, Ruiqing Cai, Ming Xia, Ming Tan, Lijuan Yuan
Combined live oral priming and intramuscular boosting regimen with Rotarix and a nanoparticle-based multivalent rotavirus vaccine confers significant protection against both G4P[6] and G1P[8] human rotavirus infection in gnotobiotic pigs

P46 Abdullahi Jamiu, Ivan Akhrymuk, Kenneth Foreman, Dmitri Klimov, Mikell Paige, Kylene Kehn-Hall
Exploiting virus-host interactions to develop novel inhibitors against Venezuelan equine encephalitis virus

P47 Nicole Sugai, Stephen Werre, Julie Cecere and Orsolya Balogh
Optimizing centrifugation parameters for cooled canine semen processing

P48 Peixi Chang, L. Yang, B. Sallapilli, and Y. Zhang
Zika virus recruits an importin to its replication site as a proviral factor

P50 Anna Zimina
Development of a novel approach to broadly-protective anti-enterovirus vaccines

P52 Urmil Dave

SESSION SCHEDULE

POSTER SESSION #2: BLOCK 1
2:00 PM - 2:45 PM
WATERFALL ROOM

P1 SI Barth, AR Wilkinson, SM DeMonaco, KM Boes, and BJ Conner

ADAMTS13 activity in dogs with chronic enteropathies

P3 Tosin Ogunmayowa, Alicia Lozano, Alexandra Hanlon, Natalie Cook, Freddy Paige, Charlotte Baker

Historical redlining and contemporary racial disparities in sports and recreation-related injury hospitalizations in the United States

P5 Amir Mortazavigazar, Ryan Calder

Causal Inference to Scope Environmental Impact Assessment in MultiSector Systems: The Case of Trans-Border Hydropower Exports

P7 Holly A. Morrison, Audrey Rowe, Kristin Eden, Daniel E. Rothschild, Katherine Baumgarner, Stephan Brown, Eda Holl, Irving C. Allen

Deletion of NIK Increases Susceptibility to Colorectal Cancer through Diminished Intestinal Epithelial Cell Regeneration

P17 Cody Swilley, Yuze Zheng, Lin Yu, Xiguang Xu, Min Liu, Kurt Zimmerman, Hehuang Xie

Sex Linked Growth Disorder and Aberrant Pituitary Gene Expression in Nestin-Cre Mediated Egr1 Conditional Knockout Mice

P19 J. Jing, X. Wang, and M. Theus

Regulation of peripheral-derived immune cells in the remodeling of pre-existing pial collateral vessels following ischemic stroke

P21 Tristan Stoyanof, Clay Caswell

A Trp with Brucella abortus

P23 Maria Buman Ruiz Diaz

MinION Next-Generation Sequencing for Rapid Identification and Distinction of PRRSV Wildtype and Vaccine Strains

P33 L Helber, A Hay, B Wagner, C Leeth, T LeRoith, T Cecere, K Lahmers, F Andrews, S Werre, A Johnson, C Clark, N Pusterla, S Reed, D Lindsay, S Taylor, Krista E. Estell, M Furr, R Mackay, FD Piero, M Carossino, K Pandaleon, S Weatherford, S Witonsky.

Persistance of Sarcocystic Neurona and Histopathologic Changes in Horses with EPM

P35 Ehab A. Salama, Yehia Elgammal, Aruna Wijeratne, Mohamed N. Seleem

Lansoprazole potentiates amphotericin B activity against multi-drug resistant Candida auris targeting cytochrome bc1 complex

P37 Yehia Elgammal, Ehab A. Salama, Mohamed N. Seleem

Hope on the horizon: HIV protease inhibitor, atazanavir overcomes azole resistance in Candida auris infection

P39 Catherine Jula, Jennifer Davis, Harold McKenzie III

Single Dose Pharmacokinetics of Pimobendan in Healthy Adult Horses

SESSION SCHEDULE

POSTER SESSION #2: BLOCK 2
2:45 PM - 3:30 PM
WATERFALL ROOM

P9 Brittany Heath, Kylene Kehn-Hall
The role of glial inflammatory mediators on neural dysregulation induced by SARS-CoV-2 infection

P11 Razan Alajoleen, Andrea Daamen, Prakash R. Timilesena, Song Li, Peter E. Lipsky, Xin Luo
Single-Cell RNA Sequencing Reveals Disease Stage-Dependent Transcriptomic Profiles of Regulatory B Cells in Systemic Lupus Erythematosus

P13 Caitlin Armstrong, Ran Lu, Xavier Cabana-Puig, Teresa Southard, Tanya LeRoith, Michael Appiah, Fernando Gutierrez Garcia, Razan Alajoleen, James C. Testerman, Liwu Li, Christopher M. Reilly, Xin Luo
A High-Fat Diet Promotes Lupus Nephritis in Lupus-Prone Mice in a Sex- and CX3CR1-Dependent Manner

P15 Dao Xu and Christopher M. Reilly
HDAC6 knockout mitigates pristane-induced lupus

P25 Nicolas Burns, Ehab Salama, Mohamed Seleem
Counteracting Aspergillus species antifungal resistance via combinational therapies

P27 Pallavi Rai, Jeffrey M. Marano, Sheryl Coutermarsh-Ott, James Weger-Lucarelli
Using mouse coronavirus to determine the impact of obesity on coronavirus disease severity and identify biomarkers of severe disease outcome

P29 Christina Pacholec, Nikolaos Dervisis, Hehuang Xie, Kevin Lahmers, Priscila Beatriz da Silva Serpa, Kurt Zimmerman
Can deep learning (CNN) detect minimal residual disease (MRD) in dogs treated for lymphoma?

P31 Nour Alkashef, Mohamed Seleem
Ritonavir, A promising cost-effective amphotericin synergist against cryptococcal meningitis infection

P41 Dima Hajj Ali, and Rajshekhar Y. Gaji
Functional Characterization of TgTKL1 Kinase in Toxoplasma gondii Pathogenesis

P43 Padmaja Mandadi, Rajshekhar Y. Gaji
Characterization of transcription factors in acute toxoplamosis

P45 Laura Bruckner
Neutrophils in the Tumor Microenvironment

P51 Xiguang Xu, Hehuang"David" Xie I
EGR1 mediates distinct programs of gene expression regulation in excitatory and inhibitory neurons

P53 Yu Lin, Hehuang"David" Xie
Spatiotemporal multi-omics analysis of the hippocampal-amygdala circuit during contextual fear memory consolidation

P54 Mashed Arabi, Hehuang"David" Xie
Single Cell Multi-Omics Analyses Reveal Mouse Hippocampal Gene Expression Aberrations with Excess Maternal Folate Supplementation

ABSTRACTS

P1
ADAMTS13 activity in dogs with chronic enteropathies

SI Barth, AR Wilkinson, SM DeMonaco, KM Boes, and BJ Conner
Department of Small Animal Clinical Sciences

Background: Chronic enteropathies (CE) predispose dogs to thromboembolic disease, but the underlying mechanisms are poorly understood. Humans with CE have decreased activity of ADAMTS13, a von Willebrand factor (vWF) cleaving enzyme, and increased circulating vWF. The primary aim of this study is to determine whether dogs with CE have reduced ADAMTS13 activity, increased plasma vWF antigen concentration (vWF:Ag), and increased vWF:collagen binding activity (vWF:CBA) compared to healthy control dogs. Hypothesis: Dogs with CE have reduced ADAMTS13 activity, increased vWF:Ag, and increased vWF:CBA compared to healthy control dogs. Methods:In this prospective study, ADAMTS13 activity, vWF:Ag, and vWF:CBA will be assessed in 20 client-owned dogs with CE. Forty healthy dogs will serve as controls for ADAMTS13 activity. Twenty healthy dogs will serve as controls for vWF:Ag and vWF:CBA. The mean ADAMTS13 activity, vWF:Ag, and vWF:CBA of the control group will be compared with the affected group using a two-sample t-test, or if the data is not normally disturbed, a Wilcoxon rank sum test. Preliminary results: Sixteen dogs with CE have been enrolled at this time. The mean age of this population is 5.9 years (standard deviation (SD) of 3.7). Mean ADAMTS13 activity is 74.5% (SD 22.6) in affected dogs (n = 9) and 83.9% (SD 14.2) in healthy dogs (n = 36). Mean vWF:Ag is 109.8% (SD 41.4) in affected dogs (n = 7) and 159.8% (SD 33.4) in healthy dogs (n = 17). Mean vWF:CBA is 97.9% (SD 42.2) in affected dogs (n = 7) and 129.8% (SD 25.9) in healthy dogs (n = 17). Preliminary analysis revealed that ADAMTS13 activity is not significantly different between dogs with CE and healthy dogs (P = 0.12). However, vWF:Ag and vWF:CBA are significantly decreased in dogs with CE compared to healthy control dogs (P = 0.005 and P = 0.032, respectively).

Support: Internally funded

P2

Time is Brain: Stimulating Tie2 Improves Pial Collateral Growth and Reduces Tissue Damage After Ischemic Stroke

Alexandra M. Kaloss[1], Kennedie Lyles[2], Nathalie A. Groot[1], Jackie Zhu[3], John B. Matson[3], and Michelle H. Theus[1,2]

[1]Department of Biomedical Sciences and Pathobiology, [2]School of Neuroscience, [3]Department of Chemistry, Virginia Tech, Blacksburg VA

Strokes are a leading cause of death and disability, with most cases being ischemic strokes resulting from blood flow in the brain being interrupted. Immediately following this vascular obstruction, often due to a blood clot, the cells in the affected region of the brain begin to die due to lack of oxygen and nutrients, resulting in neurological impairments. Treatments for ischemic strokes currently focus on removing the clot but can fail to restore blood flow and prevent cell death. Thus, novel treatments are needed that specifically target the restoration of blood flow. Specialized pre-existing arterioles, termed pial collaterals, can remodel into larger vessels after an ischemic event allowing them to ease the loss of blood flow and prevent cell death. However, the process for pial collateral remodeling remains poorly understood. Using a permanent middle cerebral artery occlusion (pMCAO) model of ischemic stroke, our previous work has shown that loss of EphA4, a receptor tyrosine kinase, results in reduced infarct volume which correlated with increased collateral size from 4.5 to 24 hours post-pMCAO. The collateral vessels lacking EphA4 also displayed significantly higher endothelial cell proliferation and immune cell recruitment at 24 hours post-stroke. We hypothesized that EphA4 was exerting its function through the inhibition of Tie2, a protein involved in vascular stability and growth. Therefore, we employed Vasculotide (VT), an Angiopoetin-1 memetic peptide, to stimulate Tie2 signaling. In vitro, endothelial cells treated with VT displayed improved migration and wound healing. Moreover, in an in vivo mouse model, animals that received intravenous VT directly following a surgically induced stroke had significantly lower infarct volumes at 24 hours post-pMCAO compared to vehicle controls. This decreased infarct volume correlated with improved performance by VT-treated mice on sensory and motor behavior tests, including adhesive tape test, rotarod, and modified neurological severity score in the first 28 days post-pMCAO, compared to controls. VT-treated mice also displayed significantly larger pial collateral vessels 24 hours post-stroke compared to vehicle-treated control mice. Therefore, inhibiting EphA4 or stimulating Tie2 could serve as novel therapeutic strategies for improving collateral response following ischemic stroke.

Support: NINDS R01NS112541 (MHT) and Office of Research and Graduate Studies

ABSTRACTS

P3

Historical redlining and contemporary racial disparities in sports and recreation-related injury hospitalizations in the United States

Tosin Ogunmayowa[1], Alicia Lozano[2], Alexandra Hanlon[2], Natalie Cook[1], Freddy Paige[3], Charlotte Baker[1]

[1]Department of Population Health Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Tech
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Approximately 9 million people are injured annually from sports and recreation in the U.S., more than a third seek treatment in the emergency department, and several thousands are hospitalized for more severe injuries. In this study, we examined the association between historical redlining, a government-sanctioned racial discriminatory practice of the 1930s, and present-day sports and recreational injury (SRI) hospitalization in the U.S. We obtained 2011 SRI hospitalization data in the U.S. from the National Inpatient Sample (NIS) database, linked it to 1930s Home Owners' Loan Corporation (HOLC) redlining maps, and assigned U.S. hospitals to one of three HOLC grades (A+B – best/still desirable, C – definitely declining, and D – hazardous/redlined). Generalized linear mixed models, accounting for sample weight, stratified sampling, and patients clustered within hospitals, were used to examine these relationships. We found no association between HOLC grade and the risk of hospitalization for SRI among all racial/ethnic groups after adjusting for confounders; however, HOLC grade was associated with length of hospital stay (LOS) in Black, Hispanic and White patients, and total hospital charges per discharge for Black and Hispanic patients. Black patients who were hospitalized for SRI in historically redlined neighborhoods (grade D) had 38% longer LOS compared to those hospitalized in neighborhoods historically graded as "A+B: best/still desirable". In contrast, White and Hispanic patients who were hospitalized for SRI in historically redlined neighborhoods had 8% and 9% shorter LOS, respectively, compared to those hospitalized in "A+B: best/still desirable" neighborhoods. Total hospital charges per discharge were 29% and 12% lower for Black and Hispanic patients hospitalized in historically redlined neighborhoods compared to those hospitalized in neighborhoods historically graded as "A+B: best/still desirable", but no difference was observed among White patients. We also found that as the social vulnerability (i.e., the susceptibility of communities to the adverse impact of injury, disease outbreaks, and natural or human-caused disaster) of present-day neighborhood environment decreased, the impact of redlining on LOS weakened for Black patients but not for Hispanic and White patients. This study indicates that redlining, an indicator of structural racism, has a lasting impact on the length of stay and cost of hospitalization for SRI in the U.S.

Support: GPSS Graduate Research Development Program, Office of Research and Graduate Studies

P4

Mechanical High-Intensity Focused Ultrasound (Histotripsy) in Dogs with Spontaneously Occurring Soft Tissue Sarcomas

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High-intensity focused ultrasound (HIFU) is a non-invasive technique able to ablate tissue by either thermal or mechanical means. Histotripsy is a non-thermal HIFU ablative therapy that causes mechanical disruption of tissue. Prior rodent studies have demonstrated that HIFU is immunogenic. Soft tissue sarcomas (STS) are a common form of cancer in dogs with biological behavior similar to humans. Adequate local tumor control in veterinary medicine may require extensive surgical resection. There is need for alternative therapies. Our objective was to investigate the in vivo feasibility of ablating STS in client-owned dogs with histotripsy and to characterize the impact of acute immunologic response. Methods: CT of the chest/abdomen/tumor was performed for staging and treatment-planning. Partial tumor ablation was performed using a prototype histotripsy system. Anatomical ablation zones were evaluated with CT at 1- and 4-days post-treatment, with tumor resection at 4-days post-treatment. Safety was monitored with exams, owner reports, and bloodwork. TME gene expression was evaluated with the Nanostring Canine IO panel. Multiplex serum cytokine levels were used to evaluate systemic immune response. TME was evaluated by characterizing changes in the infiltration of TAMs and TILs using mIHC panels. Results: Ten dogs were recruited and treated. Tumor histologies included all STS of various subtypes and grades. Differential gene expression analysis identified 79 genes with at least 2-fold upregulation between treated and untreated groups. Genes associated with inflammation, stress, immune cell migration and interactions were the highest upregulated. Myeloid compartment, NK cell functions, and interleukin gene sets obtained the highest significance score. There were no statistically significant differences in plasma cytokine concentrations between groups. In more than half of all examined samples, mIHC investigating macrophage populations showed mild to marked increases in IBA-1 and CD206 double-positive cells, representing M2-polarized macrophages. There were no significant differences in lymphocyte staining between groups. Conclusions: Histotripsy can achieve safe and effective tumor ablation in dogs with STS. Changes in the tumor microenvironment reflect increases in the expression of genes associated with inflammation, matrix remodeling, and immune cell interactions. Histotripsy as an immunotherapeutic treatment option needs to be further investigated.

Support: Focused Ultrasound Foundation

P5

Causal Inference to Scope Environmental Impact Assessment in MultiSector Systems: The Case of Trans-Border Hydropower Exports

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Decarbonization of the United States' electricity sector will require trillions of dollars of investment in generation and transmission infrastructure. The National Environmental Policy Act (NEPA) requires proponents of many major projects to complete environmental impact statements (EIS) that address reasonably foreseeable impacts, regardless of where these impacts occur. There has been controversy over the cause-effect relationships among electrical supply, electrical demand, apparent cost, and other variables given the complex interactions between them. Therefore, the range of environmental impacts attributable to new infrastructure projects is subject to frequent disagreements. In this work, we address increasing U.S. imports of Canadian hydropower in the context of falling prices and surplus generation. There has been controversy as to whether new transmission capacity stimulates new generation capacity and, thus, whether generation-side environmental and health impacts must be assessed in the scope of incremental transmission projects. We have developed a rich longitudinal database of variables related to generation capacity, export volume, retail prices, and climate over the period 1979–2021. We have applied a novel multivariable, wide neural network machine learning methodology to evaluate alternative causal models for the evolution of the electricity system and the role of new transmission infrastructure. We find no evidence that transmission capacity stimulates generation capacity. Rather, generation capacity growth in Canada is triggered primarily by domestic price signals and climate parameters, with trans-border transmission capacity developed primarily to absorb excess generation potential. This work supports a relatively narrow scope for EIS related to trans-border transmission projects. More generally, this analysis demonstrates how causal inference methods may help build consensus around the appropriate scope of EIS for highly interconnected energy and infrastructure projects.

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P6

Non-essential role for Cx3cr1-expressing EPH receptor A4 in a murine model of TBI

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Erythropoietin-producing human hepatocellular (Eph) receptors contribute significantly to central nervous system injury. Our findings demonstrated that Cx3cr1-expressing cells within the perilesional cortex showed increased levels of EphA4 after induction of controlled cortical impact (CCI) injury in mice. Cx3cr1 is a fractalkine receptor, commonly expressed on resident microglial and peripheral-derived macrophage (PDM) cells. The aim of this study is to identify the role of microglial-specific EphA4 in CCI-induced damage. Cx3cr1CreER/EYFP knock-in/knock-out mice expressing EYFP in Cx3cr1+ cells were used to evaluate microglia in EphA4-deficient mice following 1-month tamoxifen injections. CCI-Injured wild-type (WT) Cx3cr1CreER/EYFP/EphA4+/+ mice displayed increased EphA4 expression on the EYFP-positive cx3cr1 cells within the peri-lesion. Immunohistochemical applications were further used to differentiate between the peripheral-derived macrophage and resident microglia using anti-Ccr2, which selectively labeled PDMs and not microglia. We then exploited GFP bone marrow chimeric mice to discriminate EphA4 expression on microglia (TMEM119+/GFP-) versus PDMs (GFP+) following CCI. Finally, the use of Cx3cr1CreER/EYFP/EphA4f/f (KO) mice, which show no detectable transcript for EphA4 in microglia only, demonstrated no discernible difference in lesion volume or blood brain barrier (BBB) disruption when compared to the WT mice. These findings illustrate that although EphA4 is upregulated on cortical microglia after TBI, it plays a nonessential role in acute response following TBI.

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P7

Deletion of NIK Increases Susceptibility to Colorectal Cancer through Diminished Intestinal Epithelial Cell Regeneration

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Patients with a medical history of Inflammatory Bowel Disease are predisposed to colorectal cancer with greater than 20% developing colitis-associated colorectal cancer (CAC). Diminished regulation of intestinal epithelial cell (IEC) proliferation is a critical feature in the development of colorectal cancer. However, loss of stem cell regulation in crypts is widely predicted to be the cell-of-origin for malignant transformation. We hypothesize that NIK and proper noncanonical NF-κB signaling is essential for protection against colorectal cancer and functions through the epithelial cell compartment rather than the stem cell compartment. Here, we show that NF-κB inducing kinase (NIK) attenuates colorectal cancer through the regulation of IEC regeneration and differentiation mediated by noncanonical NF-κB signaling. Murine models with IEC-specific NIK deletion have increased susceptibility to inflammation-induced tumorigenesis following the chemical induction of AOM/DSS. Mechanistic studies using crypts and organoids further revealed an imbalance of proper apoptosis and proliferation signaling, with colonic crypts having increased accumulation of mature, non-dividing IECs. This suggests a model for Top-down tumorigenesis as terminally differentiated IECs lack proper turnover and compensate for decreased regenerative capacity from the stem cell niche by acquiring stem-cell like features. Our work has clinical relevancy as human CAC biopsy samples have attenuated noncanonical NF-κB signaling, including significantly decreased gene expression of NIK (MAP3K14) and effector chemokines produced (CXCL12, CXCL13, CCL19, CCL21). Here, we present a novel role for noncanonical NF-κB signaling in regulating IEC regeneration and differentiation in protecting against CAC development.

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P8
The T2-FLAIR mismatch sign as an imaging biomarker for canine oligodendrogliomas

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Background: The T2-FLAIR mismatch sign (T2FMM) is an MRI imaging biomarker of human IDH1-mutated, 1p19q non-codeleted low-grade astrocytomas. It is characterized by the presence of a homogeneously hyperintense tumor signal on T2W images and a hypointense signal with a hyperintense peripheral rim on FLAIR images. On histopathology, the T2FMM is correlated with the presence of microcysts in the mismatch regions. In canine gliomas, the T2FMM has not been described Hypotheses/Objectives: The T2FMM in canine brain MRI studies will be associated with the low-grade astrocytoma phenotype and the presence of microcysts on histopathology. Interobserver agreement will be high for the qualitative assessments of the T2FMM. Animals: 186 dogs with brain MRI evidence of focal intra-axial brain lesions;146 histologically confirmed intracranial gliomas (90 oligodendrogliomas, 47 astrocytomas, 9 undefined gliomas), 33 cerebrovascular accidents, and 7 with inflammatory lesions Methods: Two blinded raters evaluated all sequences from 186 canine MRI brain studies to identify cases with the T2FMM. Interobserver agreement was determined for qualitative MRI features defining the T2FMM by calculating intraclass correlation coefficients (ICC). Fisher exact tests were used to compare the proportions of binary MRI features present in dogs with and without the T2FMM. Immunohistochemistry for the IDH1 (R132H) mutation and gene expression analyses (Illumina) of oligodendrogliomas with and without T2FMM were performed to examine genotypes associated with this sign. Results: T2FMM was identified in 14/186 (8%) canine brain MRI, and was exclusively detected in oligodendrogliomas (14/14; 100%). Interobserver agreement for detecting T2FMM was good (ICC>0.81). The presence of the T2FMM was significantly associated with the low-grade oligodendroglioma (LGO) phenotype ($p < 0.001$), absence of tumor enhancement on post-contrast images ($p=0.002$), and microcysts or myxoid lakes on histopathology ($p <0.00001$). In oligodendrogliomas with the T2FMM, IDH1-mutations or specific differentially expressed genes were not identified. Conclusion and clinical importance: T2FMM can be readily identified on routinely obtained MRI sequences. T2FMM is a specific biomarker for canine oligodendroglioma, and was significantly associated with non-enhancing LGO.

P9

The role of glial inflammatory mediators on neural dysregulation induced by SARS-CoV-2 infection

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Neurological effects due to post-acute sequalae of SARS-CoV-2 infection (neuro-PASC) will impact public health spanning decades. Alterations in the central nervous system (CNS) have been observed in a subset of COVID-19 patients presenting with neurologic manifestations akin to Alzheimer's Disease and Parkinson's Disease. Millions of people worldwide are currently affected by a neurodegenerative disease with the number of cases expected to rise. The causal relationship of COVID-19 and neurodegenerative diseases remain to be determined, yet it is critical to promptly identify the mechanisms of neural dysfunction due to neuro-PASC. To address this gap, we aim to identify pathways in which SARS-CoV-2 infection leads to glial activation and subsequent neuronal injury and/or death. Here, we tested the direct infection of human astrocytes, microglia, and neuroblastoma cells by SARS-CoV-2. The release of infectious virus, absolute quantification of viral RNA, and fold changes in gene expression were measured. Astrocytes infected with SARS-CoV-2, maintained constant viral titers (PFU/mL) and intracellular viral RNA up to 48 hours post-infection (hpi). In contrast, microglia rapidly cleared SARS-CoV-2 infectious virus at 48hpi and intracellular viral RNA decreased at 24hpi. Next, we pre-screened cellular RNA for three markers of inflammation using RT-qPCR. CXCL10, IL1 , and IL6 were chosen due to the elevated circulating cytokines in patient serum, and due to their strong correlation with disease severity and clinical prognosis in COVID-19 patients. In astrocytes, significant increased expression of CXCL10 and IL6 was observed at 9hpi and 24hpi, respectively. Microglia exhibited significant upregulation of CXCL10 at 72hpi in absence of infectious virus. Direct infection of neuroblastoma cells with SARS-CoV-2 did not impact cell viability as determined by CellTiter-Glo. Our findings validate SARS-CoV-2 ability to modulate glial activity in the presence or absence of infectious virus. Ongoing studies aim to identify secreted inflammatory markers from SARS-CoV-2 infected glial cells and their relation to neuronal viability as determined by multiplex-ELISA and CellTiter-Glo, respectively.

Support: Office of Research and Graduate Studies

P10
Comparison of semi-automated and automated diffusion tensor imaging scalar value acquisition in dogs

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Accurate assessment of spinal cord injury (SCI) severity improves the ability of veterinarians to develop an appropriate clinical treatment plan and discuss prognosis with clients. Quantitative imaging surrogates of myelin and axonal integrity not routinely performed provide more detailed information of SCI than routine imaging and neurologic exam. Quantitative magnetic resonance imaging metrics (qMRI) previously explored for use in veterinary medicine include fractional anisotropy, apparent diffusion coefficient, mean, axial, and radial diffusivity, and magnetization transfer ratio. A limitation for including qMRI metrics in routine imaging is the wide range of variability in reported normal values. Factors involved in data acquisition of qMRI include acquisition parameters, analytic methods, and region of interest selection variability. The implementation of automated data acquisition through open-source software, such as Spinal Cord Toolbox (SCT), will aid in reducing variability of qMRI metrics due to reduced variability in region of interest placement and wide-spread software access by various institutions and clinical practices. Due to anatomic differences between human and canine vertebral columns and spinal cords, a canine atlas was created for use with SCT to improve the accuracy of automated qMRI data acquisition. Five medium size healthy dogs had spinal MRI performed from the foramen magnum caudally to the conus medullaris. The MRI data and Digital Imaging and Communications in Medicine (DICOM) images were shared with collaborators at the University of Montreal, and an atlas was created for use with SCT. qMRI ranges of fractional anisotropy, mean diffusivity, axial diffusivity, radial diffusivity, magnetic transfer ratio, and grey matter to white matter intensity ratio will be processed using SCT in the normal dogs. Data acquisition from automated region of interest (ROI) placement and automated region of interest placement with manual adjustment (semi-automated) values will be compared, and intra-operator and inter-operator variability will be assessed.

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P11

Single-Cell RNA Sequencing Reveals Disease Stage-Dependent Transcriptomic Profiles of Regulatory B Cells in Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease associated with abnormal activation of immune cells. Regulatory B (Breg) cells are a B cell subset that negatively regulate immune responses via secretion of immunoregulatory cytokines interleukin (IL)-10, IL-35, and transforming growth factor (TGF)-β. We had previously shown that in lupus-prone MRL/lpr mice, pre-disease Breg cells were more potent in suppressing autoimmunity than active-disease Breg cells. In this study, using single-cell RNA (scRNA) sequencing, we profiled ~10,000 Breg cells from female MRL/lpr mice at the pre-disease (6-8 weeks of age) vs. active-disease (10-12 weeks of age) stages. Based on known markers of Breg subsets, scRNA analysis identified respective clusters as transtional-2 marginal zone precursor B cells (T2-MZP), marginal zone B cells (MZB), transitional 1 B cells (T1), germinal center B cells (GC), and B1 B cells. Our data showed that the pre-disease Breg cells were predominantly T2-MZP and MZB cells. Follicular B cells outside the marginal zone, on the other hand, are significantly increased in the active-disease stage. Two long noncoding RNAs (lncRNAs), Malat1 and Xist, were significantly increased in active-disease Breg cells, where the expression of Lglas3, Slpi, and Spp1 was also increased. Notably, active-disease Breg cells exhibited an exhausted phenotype with increased expression of T-bet (Tbx21). Collectively, our findings suggest that the SLE disease stage-dependent functions of Breg cells are regulated at the transcriptional level. In future experiments, we will determine whether the immunosuppressive functions of active-disease Breg cells can be restored by knocking down the differentially overexpressed genes.

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P12

HDM2 inhibitor NVP-CGM097 significantly reduces alphavirus replication in microglia

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Eastern and Venezuelan equine encephalitis virus (EEEV and VEEV) are new world alphaviruses that can cause mild flu-like illness which can progress into severe neurological deficits in both humans and equines. The primary virulence factor of these viruses, the capsid protein has a variety of functions including binding to membrane glycoproteins, inhibiting transcription of host cells, and blocking nucleocytoplasmic transport. Interestingly, the capsid protein of some other encephalitic viruses, including West Nile virus, has been demonstrated to mediate apoptosis through the p53/HDM2 cell apoptosis pathway. The role of HDM2 specifically has only recently been highlighted as a contributing factor impacting viral proliferation in non-oncogenic viruses. We hypothesize that HDM2 is a pro-viral factor which is activated during EEEV and VEEV infection with the goal of establishing HDM2 as a promising antiviral target against encephalitic alphavirus replication. Here, we investigated the antiviral potential of an HDM2 inhibitor, NVP-CGM097. Treatment of microglia cells (HMC3s) with NVP-CGM097 at nontoxic concentrations (>80% cell viability) resulted in up to 4 log10 reduction in viral titers of VEEV TC-83. Additional studies revealed that treatment of EEEV (FL93-939) infected microglia with NVP-CGM097 at nontoxic concentrations resulted in a >1log10 reduction of viral titers. LC-MS/MS analysis of capsid immunoprecipitated samples from VEEV infected cells identified p53 as a potential host interactor of VEEV capsid. Furthermore, Co-immunoprecipitation with a VEEV TC-83 V5 tagged capsid verified that there is an interaction between HDM2 and VEEV capsid protein as well as p53 and VEEV capsid protein. Further studies are aimed at determining the impact of VEEV capsid on HDM2 expression and the importance of HDM2 to viral proliferation. Ultimately, this study highlights the potential for HDM2 inhibition as an antiviral against encephalitic viruses.

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P13

A High-Fat Diet Promotes Lupus Nephritis in Lupus-Prone Mice in a Sex- and CX3CR1-Dependent Manner

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The pathogenesis of SLE, which affects multiple organs systems, is poorly understood. Lupus nephritis (LN) is a common complication of SLE that occurs when there is kidney involvement. SLE, like many autoimmune conditions, has a strong female sex bias of disease incidence. Previous work in our lab indicated a protective role of a chemokine receptor, CX3CR1, in the pathogenesis of SLE using female mice from a well-characterized murine model of SLE (MRL/lpr). Here, a littermate breeding strategy was used to generate male and female MRL/lpr mice with either Cx3cr1+/+ or Cx3cr1+/- genotypes. Animals from each sex/genotype category were fed either an inflammation-promoting high-fat diet (HFD) or a control diet (CD). We hypothesized that a HFD promotes LN in MRL/lpr mice in a sex-and CX3CR1-dependent manner. Weekly assessment of proteinuria levels revealed higher proteinuria levels in female Cx3cr1+/- mice fed a HFD compared with diet- and genotype-matched controls. Proteinuria levels in male mice were unaffected by both Cx3cr1+/- genotype and diet. Similarly, gross organ weights of both kidneys and renal lymph nodes were increased for female mice in the HFD diet condition compared with the diet-matched controls. No effect of Cx3cr1+/- genotype was observed for either sex on either gross organ weight. Scoring of formalin-fixed, paraffin-embedded sections from the kidneys of female mice established an interactive effect of HFD diet and Cx3cr1+/- genotype on multiple histopathology indices of glomerular and tubulointerstitial damage. Collectively, these data support our hypothesis. Additional work is needed to determine the underlying mechanism(s) driving the observed effects.

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P14

Rift Valley fever virus NSs protein interacts with LC3 family members to modulate autophagy

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Rift Valley fever virus (RVFV) is a viral zoonosis that causes severe disease in ruminants and humans. Impregnated ruminant infections are characterized by 'abortion storms' in which abortions occur in nearly 100% of cases. In humans, RVFV causes a variance of clinical symptoms ranging from a flu-like illness to hemorrhagic fever, neurological deficits, and encephalitis. RVFV is mainly transmitted via mosquito bite and concerns are rising regarding the introduction of RVFV to the U.S. as competent mosquito vectors were identified. Despite its pathogenic potential, there are no FDA-approved therapeutics or vaccines to challenge the global spread of this infectious organism. The nonstructural small (NSs) protein is the main virulence factor of RVFV, making it an attractive antiviral target. Bioinformatic and structural analysis identified four potential LC3-interacting region (LIR) motifs in the RVFV NSs protein, suggesting that NSs forms polyvalent interactions with LC3, the host key autophagy protein. Autophagy is a homeostatic process in which cellular materials are degraded and it can act in either a proviral or antiviral manner. To determine whether NSs interacts with LC3-family proteins, isothermal titration calorimetry (ITC) experiments were performed with peptides corresponding to the predicted LIRs. ITC demonstrated that LIR4 interacts with high affinity with all six LC3 proteins, whereas weak or no binding was observed with LIR1-3. To confirm the NSs-LC3 interaction, plasmids encoding LC3-family members were utilized, and co-immunoprecipitation confirmed that NSs interacts with all six LC3-family members in RVFV-infected cells. NSs also co-immunoprecipitated with endogenous LC3A and LC3B in RVFV-infected cells. Substitution of key amino acids in LIR4 of NSs resulted in significant loss of binding to LC3B in infected cells, further indicating a crucial role for LIR4. Cellular fractionation followed by co-immunoprecipitation demonstrated the NSs-LC3 interaction occurred predominantly in the nucleus. Confocal microscopy demonstrated that NSs colocalized with LC3A in perinuclear and filamentous regions, suggesting NSs is sequestering LC3A in the nucleus to prevent antiviral autophagy. This is supported by experiments demonstrating that NSs downregulates autophagy. These results demonstrate that RVFV NSs modulates autophagy through interaction with LC3-family proteins, providing another mechanism that RVFV NSs dampens the host antiviral response.

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P15

HDAC6 knockout mitigates pristane-induced lupus

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Systemic lupus erythematosus (SLE) is an autoimmune disease often occurred in women. SLE is characterized by extreme production of pathogenic antibodies and overmany inflammation. Histone deacetylase 6 (HDAC6) belongs to class IIb histone deacetylase. It has been demonstrated that selective HDAC6 inhibitor suppresses inflammation in lupus-prone mice. It is known that C57BL/6 mice develop lupus-like disease following pristane injection. Here in this study, sex and age-matched wild type and HDAC6 knockout mice on the C57BL/6 background were selected and they were injected with 0.5 ml pristane or PBS intraperitoneally (i.p.) at 8 weeks of age. 10 days later, the mice were euthanized. At the endpoint, we measured body and spleen weight, collected the serum, and harvested peritoneal cells and splenocytes for flow cytometry. We found pristane treatment increased the spleen weight and there was no difference between WT mice and HDAC6 knockout mice. Moreover, there was no difference in T cell or B cell populations in the splenocytes as shown by flow cytometry results. Pristane treatment motivated the population of inflammatory monocytes (CD11b+Ly6C++) and neutrophils (CD11b+Ly6G+). The recruitment of these inflammatory monocytes and neutrophils in the peritoneum was significantly decreased in HDAC6 knockout mice compared to the WT mice. In addition, pristane treatment promoted the interferon (IFN) signature genes, including Isg15, Cxcl10, Irf9, Irf7, Mx1, and Oas1a. qPCR results showed that these IFN signature genes were decreased in HDAC6 knockout mice compared to the WT mice. In brief, our study shows that HDAC6 knockout inhibits the recruitment of inflammatory monocytes and neutrophils in the peritoneum in early inflammation response to pristane. HDAC6 deletion also inhibited the expression of IFN signature genes after pristine stimulation.

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P16

NAK associated protein 1/NAP1 is required for mitosis and cytokinesis through TBK1 activation

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Successful cell division is dependent on precise and timely transition between different cell cycle phases, which is regulated by dynamic changes in protein phosphorylation status. Thus, protein kinases play a vital role to orchestrate almost every step of cell division. Impaired kinase activity often leads to abnormal cell cycle events which consequently become the underlying cause for developmental defects or abnormal cell proliferation leading to cancer. Tank Binding Kinase 1 (TBK1) is one such kinase which is often over-expressed in certain cancer types and also regulates the process of cell division. TBK1 is activated on the centrosomes during mitosis, and its loss impairs cell division resulting in growth defects and the accumulation of multi-nucleated cells. Therefore, both proper activation and subcellular localization of TBK1 are essential for mitotic progression. Yet, the upstream regulation of TBK1 during mitosis is unknown, and the function of activated TBK1 on the centrosomes is understudied. Activation of TBK1 depends on its binding to an adaptor protein which induces a conformational change leading to transautophoshorylation on serine 172 of TBK1. Our study objective is to identify the unknown upstream adaptor(s) and downstream substrates of TBK1 during mitosis. Using a combination of shRNA mediated knockdown cell lines, editing of several TBK1 associated genes via CRISPR, conditional protein degradation, and co-immunoprecipitation experiments, we identified the adaptor protein for TBK1 activation during mitosis is NAK Associated Protein1 (NAP1/AZI2). Characterization of mitotic and cytokinetic defects suggests that loss of either NAP1 or TBK1 results in the accumulation of binucleated and multinucleated cells, possibly due to several mitotic and cytokinetic defects seen in these knockout cells. We establish NAP1 as a cell cycle protein which colocalizes with activated TBK1 on the centrosomes. Interestingly, NAP1 levels during mitosis are tightly regulated by TBK1, where activated TBK1 phosphorylates NAP1 on serine 318 flagging it for ubiquitin proteasomal degradation. Further, by an unbiased quantitative phosphoproteomics analysis during mitosis, the substrates discovered reveal that TBK1 also regulates essential cell cycle kinases such as Aurora A and Aurora B. In conclusion, our work has uncovered a novel function for the NAP1-TBK1 complex during mitosis, which is distinctive from its previously known role in innate immune signaling.

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P17

Sex Linked Growth Disorder and Aberrant Pituitary Gene Expression in Nestin-Cre Mediated Egr1 Conditional Knockout Mice

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Genes regulating hormone release are essential for maintaining metabolism and energy balance. Egr1 encodes a transcription factor regulating hormone production and release, and the decreased growth hormone has been reported in Egr1 complete knockout mice. Reduction in growth hormone level has also been observed in Nestin-Cre mouse, a frequently used model to study nervous system. Currently, it remains elusive how Egr1 loss or Nestin-Cre driver disrupts pituitary gene expression. Here we compared the growth curves and pituitary gene expression profiles for Nestin-Cre mediated Egr1 conditional knockout (Egr1cKO) mice together with their controls. Reduced body weight was observed for both Nestin-Cre and Egr1cKO mice and the loss of Egr1 has slightly severer impact on females than on male mice. RNA-seq data analyses revealed that the sex-related differences were amplified in Nestin-Cre mediated Egr1 conditional knockout mice. In addition, in males, the influence of Egr1cKO on pituitary gene expression may be overridden by the Nestin-Cre driver. Differential genes associated with Nestin-Cre driver were significantly enriched for genes related to growth factor activity and binding. Altogether, our results demonstrate that Nestin-Cre and the loss of Egr1 in neuronal cell lineage have distinct impacts on pituitary gene expression in a sex-specific manner.

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P18

Transcriptomic and Proteomic Analysis of Venezuelan and Eastern Equine Encephalitis Virus Infected Astrocytes

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The mosquito-borne equine encephalitic alphaviruses, Venezuelan and eastern and equine encephalitis viruses (VEEV and EEEV), are known to cause disease in both humans and horses in sporadic outbreaks across the Americas. These viruses vary in severity of pathogenesis and sequelae, but both can lead to significant brain inflammation with long lasting debilitating neurological effects in humans. However, there is a lack of effective countermeasures available to treat these infections and knowledge regarding host processes impacted by these viruses, especially for EEEV, is lacking. Two complementary “omics” approaches, RNAseq and reverse phase protein array (RPPA), were utilized to determined host factors and pathways altered in VEEV and EEEV infected U87MG astrocytoma cells. RNAseq identified 407 and 1235 differentially expressed genes in VEEV and EEEV infected cells, respectively. EIF2 signaling, Regulation of eIF4 and p70S6K Signaling, mTOR Signaling, Protein Ubiquitination Pathway, Mitochondrial Dysfunction, Huntington's Disease Signaling, and Caveolar-mediated Endocytosis Signaling were amongst the top dysregulated pathways in both VEEV and EEEV infected cells. RPPA analysis was performed to detect alterations in the phosphorylation status or overall protein levels of 113 proteins involved in a wide array of cellular pathways including cell cycle, apoptosis, growth, inflammation, transcription, translation, autophagy, and innate immune responses. Twenty-three and 31 proteins were found to be statistically significantly altered in VEEV and EEEV infected cells, respectively. Phosphorylation of HSP27, p38 MAPK, SMAD 1/5/9, and S6 Ribosomal protein was significantly increased with both infections. In contrast, phosphorylation of Histone H3, pRB, and Aurora A were commonly downregulated by VEEV and EEEV. Currently our studies are confirmed these alterations using orthogonal assays and testing inhibitors of these pathways to determine their influence on alphavirus replication.

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P19

Regulation of peripheral-derived immune cells in the remodeling of pre-existing pial collateral vessels following ischemic stroke

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Stroke is the fifth leading cause of neurological morbidity and mortality in the United States. Approximately, 85% of which are diagnosed as ischemic resulting from arterial obstruction of the middle cerebral artery (MCA) in the pial surface of the brain. Pre-existing pial collateral vessels connect the MCA branches to the anterior or posterior arteries on both dorsal hemispheres, they represent vascular redundancies that help retrogradely re-supply cerebral blood flow to the ischemic tissue following vascular occlusion. Remodeling of pial collateral vessels (arteriogenesis) is crucial to restore blood perfusion into the ischemic penumbra and reduce tissue damage. Increased fluid shear stress induced by altered blood flow results in the activation of the endothelium and subsequent recruitment of peripheral-derived immune cells (PDIs), which triggers the outward growth of collateral vessels. While little is known about the cell-type specific contribution of PDIs in the remodeling process, previous results showed that peri-vascular macrophages are critical regulators of arteriogenesis. Our recent studies indicate that EphA4 receptor tyrosine kinase serves as a suppressor of arteriogenesis in ischemic stroke and limits the pro-resolving phenotype of monocyte/macrophages (MMs). The objective of our current study is to explore the novel role of EphA4 in regulating PDIs-mediated pial collateral remodeling in a mouse model of permanent middle cerebral artery occlusion (pMCAO). Our initial findings, using bone marrow chimeric EphA4 knockout mice and the selective arteriole labeling technique, vessel painting, demonstrate that PDI-specific EphA4 negatively regulates arteriogenesis after pMCAO. We hypothesize that selective expression of EphA4 on PDI cells, specifically MMs, influences their recruitment and functions to restrict pial collateral remodeling, cerebral blood flow restoration and tissue protection following pMCAO. Overall, these studies will give an insightful understanding of EphA4 in mediating PDI-mediated arteriogenesis after pMCAO and investigate if MMs-specific EphA4 in the peripheral circulation contributes to the acute neurovascular inflammation during arteriogenesis.

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P20
Transcriptomic Profiling of Astrocyte Development

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Astrocytes are the most abundant glial cell type in the central nervous system (CNS) and are responsible for many crucial roles in CNS homeostasis and synaptic transmission. Surprisingly little is understood regarding the gene expression changes occurring during early postnatal astrocytic development, when astrocytes undergo complex morphological and maturational changes. Understanding these developmental gene expression patterns may provide much needed insight into neurodevelopmental diseases such as Rett syndrome, autism spectrum disorder and fragile X syndrome. In the present study, we examined gene expression in wild type (WT) astrocytes at five early developmental timepoints spanning the period from astrocyte birth to maturation. Unsurprisingly, we found the highest number of differentially expressed genes (DEGs) between postnatal days (P) 14 and P28 in WT animals, a critical time in astrocyte morphological maturation. This period of time has been linked to changes in gene and protein expression associated with astrocyte maturation, as well as active astrocytic morphological maturation processes. Focusing on this developmental time period, pathway analysis of these DEGs help us identified that development, ion transporters and channels, G-protein-coupled receptors and kinase signaling cascades, cellular metabolism, and several pathways implicating cellular structure, growth, and morphology are upregulated in WT astrocytes between P14 and P28. In contrast, WT astrocytes downregulated biological pathways associated with gene and protein expression, the cell cycle, cellular metabolism (including fatty acid metabolism), and regulation of cell size at the same time point comparison. During time period, multiple pathways associated with cellular morphology and maturation functions are enriched in WT astrocytes.

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P21

A Trp with Brucella abortus

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As one of the primary building blocks in biology, amino acids can quickly become a key asset for bacteria to infect a host. Brucella abortus, an intracellular pathogen, is able to synthesize tryptophan (Trp), but it is very energetically demanding to produce. In order to be efficient, Trp biosynthesis is repressed by a variety of methods when environmental Trp levels are above a certain threshold. In these conditions, transcriptional attenuation of trpE leads to a truncated mRNA product (rnTrpL) that has new regulatory functions, including the repression of the trpDC operon. In the closely-related bacterium Sinorhizobium meliloti, rnTrpL also regulates genes unrelated to Trp biosynthesis, including those involved with quorum sensing and efflux pumps. It is unknown what regulatory targets rnTrpL has in B. abortus, and few phenotypes have been observed when trpL is deleted. Despite a lack of virulence defects in both macrophage and mouse models of infection, a growth defect in the trpL mutant was observed when grown in a defined medium with very low Trp concentrations. Although this is counterintuitive to the proposed model, current work is focused on ensuring the trpL mutation did not affect trpE expression and defining the TrpL regulon via RNA sequencing.

Support: NIH

P22

Attenuation mechanism of an interferon gamma expressing chikungunya vaccine

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Chikungunya virus (CHIKV) is an emerging pathogen that has caused millions of infections globally within the last 15 years and has the potential to become endemic in the US. CHIK disease is characterized by debilitating chronic arthritis which causes a significant reduction in quality of life. The current standard of care is based on treating symptoms and is often ineffective, and no vaccine is available to prevent disease. Furthermore, current live-attenuated CHIKV vaccine candidates in development have safety concerns. Previously, our group developed a mouse interferon-gamma expressing CHIKV-Semliki Forest virus (SFV) chimera (CHIKV-SFV/IFNγ) vaccine that was extremely safe, resulting in reduced footpad swelling and limited capacity to replicate in immunocompromised mice. Towards understanding the increased attenuation mechanism of CHIKV-SFV/ IFNγ, we have identified a subset of IFNγ-regulated antiviral genes in the guanylate binding protein (GBP) family that are highly upregulated in CHIKV-SFV/DomC-IFNγ infected fibroblasts and mice. We hypothesized that vaccine-driven IFNγ-expression stimulates these genes to restrict viral replication and thus self-contain the virulence of the vaccine. To scrutinize these genes' role in attenuation, we used siRNA to knockdown GBP gene expression or GBP overexpression plasmids prior to infection with CHIKV-SFV/IFNγ. We also created a human IFNγ-expressing vaccine to understand if these identified mechanisms could be directly applicable for human health. Lastly, we also explored the promise of IFNγ as a broadly effective antiviral against several other viruses and thus demonstrate further use of our vaccine platform. This project will define a clear role for IFNγ in modulating viral replication of CHIKV, particularly in regard to the enhancement of vaccine safety, and may identify novel strategies to improve vaccination or therapeutic development. Furthermore, the data obtained from these studies will establish a foundation to investigate the use of IFNγ against other pathogens threatening human health.

Support: Virginia-Maryland College of Veterinary Medicine

P23
MinION Next-Generation Sequencing for Rapid Identification and Distinction of PRRSV Wildtype and Vaccine Strains

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Background: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is a 15,000-base pair, single-stranded RNA virus with remarkable mutational ability. It has evaded routine control methods since it's discovery in the 1980s and is considered endemic in all US commercial swine herds, annually costing $641 million. PRRSV is classified into PRRSV-1 (European, EU) and PRRSV-2 (North American, NA) subtypes, with an indeterminate number of strains and lineages within each subtype.
The disease is commonly diagnosed by clinical signs and either antibody ELISA tests or PCR tests using Open Reading Frame 5 (ORF5), notorious for its variability amongst strains. Antibody tests cannot distinguish between naturally infected pigs and vaccinated pigs, and PCR can only differentiate EU vs NA strains, but not between their corresponding vaccines. The goal of this study is to develop a novel diagnostic test using Oxford Nanopore Technology (ONT) MinION™ next-generation sequencing (NGS) to quickly and accurately detect individual strains of PRRSV in pigs regardless of vaccine status, and potentially identify multiple genotypes in a sample.
MinION™ uses nanopore technology capable of sequencing 20 million base pairs or more within 24 hours. DNA samples are end-prepped with special targets that anchor to a gel and are ratcheted though a nanopore three nucleotides at a time, creating changes in the electrical current which are then interpreted by ONTs open-source software, Guppy™, thus creating highly accurate reads. These nucleotide sequences are then aligned and searched with a genome sequence bank such as BLAST, to identify wildtype strains, vaccine strains, or previously undescribed strains.
Hypothesis: PCR amplification paired with ONT sequencing will be able to differentiate PRRSV-2 genotypes in mixed samples.
Preliminary Conclusions: Primer pairs bound to cDNA, but not for the expected regions. We suspect this may be due to non-specific binding of the designed primers. However, we plan to continue testing with animal serum and decide if these primers may be useful for diagnostic testing. Many publications have cited the use of MinION™ sequencing for diagnostic testing, including field testing during the 2014 Ebola virus outbreak. Therefore, we presume MinION™ will be capable of detecting PCR amplicon samples and differentiating between strains within the same sample.

P24

Regulation of developmental cell death in hypothalamic corticotrophin-releasing hormone neurons through DSCAML1 and cortisol signaling

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The neuroendocrine stress axis, known as the hypothalamic-pituitary-adrenal (HPA) axis, is mediated by the hormones CRH, ACTH, and cortisol. CRH is produced in the neurosecretory area of the hypothalamus and regulates the release of ACTH, which then regulates the release of cortisol. The number of CRH-expressing neuron is precisely regulated during development, but it remains unknown what molecular mechanisms determine cell number and how cell number contributes to HPA axis function. In previous work, we found that Down syndrome cell adhesion molecule like-1 (DSCAML1), a cell adhesion molecule with links to neurodevelopmental disorders, promotes CRH neuron cell death and reduces overall CRH neuron number. In addition, DSCAML1 deficiency also results in abnormal function of the stress axis, notably the overproduction of cortisol at baseline. As cortisol has been shown to affect cell death during development, we hypothesized that DSCAML1 may affect CRH neuron cell death via the regulation of baseline cortisol. To test this hypothesis, we sought to modulate cortisol independently of dscaml1 and determine the effects on developmental CRH neuron cell death. We will use zebrafish as the model organism, as they boast a homologous hypothalamic-pituitary-interrenal (HPI) axis and the ability to easily visualize the brain in early development. To control cortisol levels, CRISPR-mediated genome engineering will be used to delete the Steroidogenic acute regulatory protein (STaR) gene, which is produced in the interrenal glands and is necessary for the synthesis of cortisol. This gene will be targeted using three (3) short-guide RNAs (sgRNAs) to introduce frameshift mutations, inhibiting STaR and consequently cortisol production. Fluorescence in-situ hybridization (FISH) will be used to label CRH neurons and immunostaining with anti-activated caspase 3 will be used to label apoptotic cells. Group-wise comparisons of cell death and CRH neuron cell number will first be made between STaR deficient animals versus sibling controls in the wild-type background. Then, we will examine STaR deficiency in the dscaml1 mutant background to determine whether dscaml1 deficiency affects CRH neuron cell death in the absence of cortisol signaling. These experiments will advance our understanding of stress axis development as well as the role of cortisol in cell death, and provide insights into stress axis-linked human neurodevelopmental and psychiatric disorders.

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P25

Counteracting Aspergillus species antifungal resistance via combinational therapies

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Aspergillus fumigatus annually contributes to more than 300,000 infections globally, with an average mortality of 65%. Invasive pulmonary infections arising from Aspergillus fumigatus are life-threatening fungal infections for immunosuppressed and cancer patients. However, the disease is much broader in scope, with cases of COVID-19 infections also containing aspergillus infections, raising the specter of more infections that are undiagnosed. Triazoles, such as voriconazole, itraconazole, posaconazole, are primary antifungals for first-line therapies for infections arising due to Aspergillus sp. Our predominant issue is the ever increasing incidence of broadly antifungal resistant strains of A. fumigatus. These incidents are and will continue to increase and trigger alarm due to treatment failure and high mortality. Mortality for these infections can range from 40-90%. In recognition of this threat alongside other fungal pathogens, the CDC has generated a watch list. Aspergillus fumigatus has been placed within the critical priority group due to its rate of occurrence, predation of the ill and rapidly growing resistance. Therefore, our group is searching for a co-drug capable of enhancing frontline antifungal potency. Here our group sought to determine the mechanisms behind interactions between HIV protease inhibitors and frontline azole drugs. We challenged various pathogenic and clinically important A. fumigatus isolates. Through this extensive investigation lopinavir was identified to work with powerful synergy when in tandem with itraconazole and posaconazole against A. fumigatus isolates. Lopinavir demonstrated synergistic relationships with itraconazole against 16 A. fumigatus isolates and 4 Aspergillus species, niger, flavus and brasiliensis; ΣFICI values were between 0.188 and 0.375. An indifference effect was noted, with an ΣFICI ranging from 0.53 to 1.125. Surprisingly, once lopinavir was in growth media with posaconazole, effective synergistic outcomes were determined to be present in 22 isolates, their ΣFICI range was 0.091 to 0.188; an indifference effect was noted in 3 azole-resistant isolates, 0.53 to 0.62. Furthermore, lopinavir has shown itself capable of re-sensitizing azole-resistant A. fumigatus isolates, 731 and 733 from the CDC. These results generate novel routes for further investigation and tenable translatable treatments that can drastically reduced the azole payload necessary to remove Aspergillus fumigatus from an infected host.

Support: NIAID

P26

Mounting a mitochondrial defense: Innate immune receptor NLRX1 regulates cell metabolism and death for protection against Lyme disease

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Lyme disease, caused by the bacterium Borrelia burgdorferi, is an emerging infectious disease of global concern. Roughly 60% of untreated patients will develop inflammation of the joints termed Lyme arthritis. This persistent phenotype results from sustained inflammatory signaling by the innate immune system. Currently, there are limited treatments for antibiotic-refractory Lyme arthritis, warranting our investigation into how the innate immune system can mitigate this inflammation. Here, we studied how the anti-inflammatory innate immune receptor NLRX1 regulates host-pathogen interactions in response to Borrelia burgdorferi. Expressed almost ubiquitously in mammalian cells, NLRX1 senses conserved genetic motifs of pathogens to regulate innate immune pathways. Specifically, NLRX1 associates with the mitochondria, playing unique roles in regulating cell metabolism and function. Because NLRX1 modulates cell function, including inflammatory signaling, cell death, autophagy, and cell metabolism, we hypothesized that NLRX1 could have unique antimicrobial defenses against Lyme arthritis. Through 30-day models of infection in novel Nlrx1-/- mice, we found that NLRX1 significantly decreased arthritis severity in wildtype mice when compared with knockouts, modulating bacterial load in vivo. We next determined in Nlrx1-/- murine macrophages that NLRX1 may control B. burgdorferi persistence by promoting Reactive Oxygen Species (ROS)-mediated oxidative stress, subsequently promoting mitochondrial dysfunction and inflammatory cell death. Finally, by infecting novel NLRX1 overexpression human monocytes, we found that elevated NLRX1 significantly decreased pro-inflammatory NF-κB cytokine secretion. These results indicate that NLRX1 plays a protective role in mitigating Lyme arthritis in both murine and human models. Further, this protection could occur through NLRX1's promotion of oxidative stress, which may initiate pore formation and inflammatory cell death in tandem with suppression of pro-inflammatory NF-κB signaling. Therefore, we emphasize the importance of NLRX1 host sensing of B. burgdorferi and encourage its further exploration for new treatments for Lyme arthritis.

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P27

Using mouse coronavirus to determine the impact of obesity on coronavirus disease severity and identify biomarkers of severe disease outcome

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Coronavirus disease 2019 (COVID-19) has been shown to cause more severe or even fatal disease in the elderly and individuals with co-morbidities. Obesity is an important co-morbidity impacting ~13% adults globally and 43% adults in the U.S. and has been associated with increased risk of hospitalizations in COVID-19 patients. Current knowledge on the impact of co-morbidities is based on epidemiological evidence and/or infections of non-natural hosts with SARS-CoV-2, but COVID-19 disease outcome is determined by complex host-virus interactions including host's immune response to the virus. Therefore, a more relevant model is needed to establish a causal link between co-morbidities and coronavirus disease severity, which can then be used to better understand coronavirus pathogenesis and to identify biomarkers associated with severe disease outcomes. To that end, we infected lean and diet-induced obese (DIO) mice with mouse hepatitis virus (MHV)-1, a mouse coronavirus, and found that obese mice had more severe disease in terms of weight loss, mortality, and lung histopathology. To identify biomarkers of severe disease, we performed RNA sequencing (RNASeq) analysis on blood samples collected at 2 days-post-infection (dpi) and these can be used in timely diagnosis and risk stratification of patients for effective utilization of health care resources.

Support: NSF

P28
Regulation of and by Quorum Sensing Proteins in the Zoonotic Pathogen Brucella abortus

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The zoonotic pathogen Brucella abortus is a causative agent of brucellosis, a potentially debilitating, persistent bacterial disease characterized by fatigue, arthritis, and periodic fever. Brucella is able to disseminate throughout the mammalian host via replication and survival in mesenchymal cells, particularly macrophages, while eliciting only a minimal immune response. How Brucella is adapted to this stealthy lifestyle, and how it regulates its genetic expression to achieve persistence in the host are major questions regarding Brucella pathogenesis. One set of virulence factors utilized by Brucella are the two quorum sensing proteins VjbR and BabR, which are master transcriptional regulators that are major determinants of Brucella's metabolic and virulence state. While strains lacking one of these two proteins have been explored, including as potential vaccines, we have created the first strain of Brucella lacking both proteins. Our data show that after removing both proteins, mice clear the resultant strain of Brucella by 4 weeks post-infection, and that the lack of both proteins has physiological consequences for how Brucella responds to host-derived stresses encountered during its infection cycle. Current work is being pursued to better define in vitro stress responses in this Brucella strain, as well as to better determine how the two proteins regulate one another in natural Brucella infections.

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P29

Can deep learning (CNN) detect minimal residual disease (MRD) in dogs treated for lymphoma?

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The focus of this study is to demonstrate that a convoluted neural network (CNN), using cytology images from dogs undergoing treatment for lymphoma, can be used to establish minimal residual disease status similar to which is typically done using molecular testing.

Support: Veterinary Memorial Fund (VMF) and American Kennel Club (AKC)

P30

Proliferation, Plasticity, and Migration: Exploring the synergistic relationship between eosinophils and TH2 cells in development of HES-like syndrome

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Eosinophils play an important therapeutic role in combating fungal and helminthic pathogens. However, aberrancies in eosinophil proliferation, activation, and chemotaxis can also lead to acute eosinophilic leukemia, paraneoplastic eosinophilia associated with T cell lymphoma and carcinomas, systemic and local hypersensitivity reactions, and Hypereosinophilic Syndrome (HES), respectively. HES is an idiopathic disease affecting humans and veterinary patients characterized by persistent eosinophilia (>1,500 cells/uL) and eosinophilic infiltration of solid organs and tissues leading to increased morbidity and mortality. Previous studies have demonstrated the role of NF-κB inducing kinase (NIK) an upstream regulator of the noncanonical NF-κB signaling pathway, in development of a HES-like syndrome due to aberrant TH2 polarization in the Nik-/- murine model. However, the intricacies of eosinophilic granulopoiesis, education of eosinophilic precursors in different hematopoietic compartments, eosinophil/neutrophil plasticity, and chemotactic ability has not been assessed during development of HES-like syndrome in Nik-/- mice. Here we show that loss of NIK potentially enhances eosinophilic chemotactic potential to eotaxin (CCL11), enhances eosinophilic/neutrophilic plasticity, and affects granulopoiesis in the bone marrow and spleen beginning at the level of eosinophilic metamyelocytes, despite potentially decreasing the proliferative potential of these cells. Taken together, these results suggest that NIK and the noncanonical NF-κB signaling pathway plays a role in dictating the hematopoietic and chemotactic potential of eosinophils and highlights a synergistic relationship with TH2 cells and their respective cytokines during development of HES-like syndrome in Nik-/- mice. Overall, these findings warrant further study to better characterize the synergistic relationship described above to better understand development of HES and HES-like diseases. Furthermore, these results may also suggest a role for NIK and the noncanonical NF-κB signaling pathway when attempting to better characterize, diagnose, and treat eosinophilic leukemias or paraneoplastic eosinophilia associated with T cell lymphoma.

Support: ICTAS, Office of Research and Graduate Studies

P31
Ritonavir, a promising cost-effective amphotericin synergist against cryptococcal meningitis infection

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Cryptococcus neoformans causing life-threatening meningitis and represents an alarming global health threat associated with high mortality among immunocompromised individuals and HIV patients. The current arsenal of antifungal drugs to combat the growing problem of cryptococcus is very limited. Amphotericin B is the front line treatment for cryptococcal meningitis. However, the treatment with amphotericin B is commonly associated with severe adverse effects. In this study, we used the combinatorial approach to minimize the toxicity and to enhance the efficacy of amphotericin B against C. neoformans. We evaluate the HIV-protease inhibitor, ritonavir, as a potential co-drug to work synergistically and to enhance the effectiveness of amphotericin B treatment. Ritonavir exhibits a potent in-vitro synergistic interactions when combined with amphotericin B against 100% (15/15) of the tested C. neoformans isolates with a fractional inhibitory concentration index (ΣFICI) ranging from 0.07 to 0.31. Notably, the combination of ritonavir with amphotericin B led to killing of all tested isolates within 3 hours as measured by time killing assays. As a part of the involved mechanistic study, ritonavir significantly interferes with glucose transport in C. neoformans reducing its uptake by 52%. These data highlight the potential of antifungal combination between amphotericin B and ritonavir to combat C. neoformans infections. Furthermore, these data will provide insight into the potential clinical usefulness of ritonavir because it is commonly administered in HIV-infected patients and cryptococcus is a leading cause of morbidity and mortality in those patients.

Support: NIH

P32

LAMPore sequencing as a novel diagnostic for equine herpesvirus 1 using equine nasal swabs

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Equine herpesvirus type 1 (EHV-1) is an alphaherpes virus that is ubiquitous in the equine population, affecting horses of every breed and discipline. While the majority of horses have been exposed by one year of age, and typically experience mild respiratory disease, infection is also associated with late term abortion, neonatal foal death, and neurologic disease- Equine Herpes Myeloencephalopathy or EHM. Test results for current detection methods, such as PCR and virus isolation, can take multiple days before they become available. A novel DNA sequencing technology, the MinION nanopore sequencer, offers a new tool to rapidly detect and differentiate EHV-1 (i.e., neurotropic and non-neurotropic strains) as a stall-side diagnostic and in field applications (e.g., naturally occurring disease and outbreaks). DNA is extracted from clinical samples, such as nasal swabs, in preparation for sequencing. Extraction of EHV-1 DNA from nasal swabs is often low yield, thus viral amplification prior to sequencing is ideal. Loop-mediated isothermal amplification (LAMP) allows for simple and rapid viral amplification. This method does not require a thermocycler nor nucleic acid purification, thus it can be done quickly with minimal equipment and will be less effected by inhibitors found in clinical samples. The purpose of our study is to compare the efficacy of LAMP and DNA sequencing using the Minion nanopore sequencer to the current gold standard qPCR for diagnosing equine herpesvirus type-1 in equine nasal swabs. Nasal swabs from Six EHV-1 positive and six EHV-1 negative horses were processed using LAMP followed by Minion sequencing. DNA from each sample was also analyzed by qPCR. Our data currently shows that LAMP and DNA sequencing with the Minion successfully identify EHV-1 in nasal swabs from horses.

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P33
PERSISTENCE OF SARCOCYSTIS NEURONA, AND HISTOPATHOLOGIC CHANGES IN HORSES WITH EPM

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EPM is a devastating neurologic disease in horses and neurologic deficits can persist long past acute disease. Until the underlying mechanisms of the immunopathology and neuropathology are better elucidated, the ability for horses to recover and remain healthy is limited. Therefore, to unravel these mechanisms, the objective of this study was to define the immunopathologic changes associated with Sarcocystis neurona infection. EPM-affected and control horses were enrolled based on our case definitions. Immunofluorescence, cytokine, and chemokine analysis, as well as immunohistochemistry (IHC) and polymerase chain reaction (PCR) for S. neurona were performed. S. neurona was identified in all EPM-affected horses (n=9), including those previously treated with anti-protozoal medications (n=4). S. neurona was identified by IHC at a significantly greater frequency than PCR. Horses chronically affected with EPM had increased degenerative histopathologic changes in their CNS compared to acutely affected horses. Degenerative changes including scarring, spheroid formation, and neuron degeneration. Based on our findings, we propose that S. neurona has the ability to persist even after antiprotozoal treatment, which may result in degenerative changes. Due to the limited case numbers with a diverse treatment history and chronicity of disease, larger studies are warranted to further define the immuno- and neuropathology associated with S. neurona infection, including assessing whether IHC for S. neurona is a more accurate diagnostic technique.

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A potent antibacterial activity against Clostridioides difficile is exhibited by antifungal drugs

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Clostridioides difficile is a prominent source of healthcare-associated infections and is regarded as an urgent public health problem globally. The only FDA-approved antibiotics for the treatment of C. difficile infection (CDI) are vancomycin and fidaxomicin. The high rate of treatment failure and recurrence linked to these antibiotics, as well as the rising number of infections, make CDI treatment extremely difficult.
Therefore, it is imperative to find new, powerful anti-C. difficile drugs. When compared to de novo drug innovation, drug repurposing is a potential technique for reducing costs and time and decreasing risks associated with de novo drug discovery. Utilizing this approach, we screened 3200 FDA-approved drugs against C. difficile, and the results showed that miconazole is a powerful inhibitor of the bacterium with a minimum inhibitory concentration (MIC) of 1 μg/ml. On the strength of this, we tested a library of 24 azoles against a diverse range of pathogenic C. difficile strains. Miconazole, econazole, and tioconazole displayed the most potent activity against C. difficile inhibiting the growth of 50% of tested isolates (MIC50) at concentrations of 1 μg/ml, 2 μg/ml, and 2 μg/ml, respectively. Miconazole was selected for further investigation since it demonstrated the most potent anti-C. difficile activity, and it is orally bioavailable. In a time-kill kinetics study, miconazole showed a fast bactericidal activity outperforming vancomycin, where it decreased a high bacterial inoculum by more than 3 log10 within 2-4 hours and completely cleared the bacterial burden after 4 hours. Physicochemical properties of miconazole including, the effects of pH, pre-exposure to simulated gastric fluid (SGF), and simulated intestinal fluid (SIF), were also examined. High pH values did not affect the miconazole's antibacterial action, and it retained the same potency after being exposed to SGF and SIF. Furthermore, miconazole did not show inhibitory activity against key species that compose the host intestinal microbiota and showed a prolonged post-antibiotic effect (PAE) (>6 hours) exceeding that of the drug of choice, vancomycin.
Overall, these results indicate that miconazole merits further investigation as a potent and selective anti-clostridial agent to replenish the dry pipeline of new anti-C. difficile therapeutics.

Support: NIH

P35

Lansoprazole potentiates amphotericin B activity against multi-drug resistant Candida auris targeting cytochrome bc1 complex

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Candida auris has emerged as a problematic fungal pathogen associated with high morbidities and mortalities. Amphotericin B is the most effective and broad-spectrum antifungal agent used for treatment of invasive fungal candidiasis with extremely rare resistance among clinical isolates. However, the clinical efficacy of this drug has been impacted recently with the emergence of C. auris which possessed extraordinary resistant profile against all available antifungal drugs, including amphotericin B. There is an urgent need for novel antifungal agents or co-drugs capable of restoring/enhancing the antifungal activity of amphotericin B and reducing its toxicity. In this study, by screening a panel of ~3,400 FDA-approved drugs we identified the proton pump inhibitor, lansoprazole, as a potent enhancer for the activity of amphotericin B against C. auris. Lansoprazole exhibited potent synergistic interactions with amphotericin B against 18/20 (90%) C. auris isolates with ΣFICI ranged from 0.25 to 0.5. Proteome Integral Solubility Alteration (PISA) assay revealed that lansoprazole inhibits an essential target in the yeast cytochrome system (Rieske protein of the mitochondrial cytochrome bc1 complex) leading to increase in the oxidative stress in the fungal cells which consequently augment the oxidative damaging effect of amphotericin B on C. auris cells. The target was confirmed with the rotenone rescue assay and transcriptome sequencing (RNA-Seq) analysis. Most importantly, lansoprazole restored the in vivo efficacy of amphotericin B in an immunocompromised mouse model, resulting in a 1.7-log (~98%) CFU reduction in the kidney burden of C. auris. In conclusion, our results identified lansoprazole as a potent enhancer to the antifungal activity of amphotericin B in addition to identification of mitochondrial cytochrome bc1 as a novel drug target to overcome the antifungal resistance in C. auris.

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Construction of novel antibiotic-resistant mutants of Neisseria gonorrhoeae strains suitable for mouse vaginal infection model

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Gonorrhea, a sexually transmitted disease caused by Neisseria gonorrhoeae, is the second most common sexually transmitted bacterial infection in the United States. The increasing prevalence of N. gonorrhoeae infections has been due to the emergence of antimicrobial-resistant strains. Most seriously, this uprising resistance to all classes of antibiotics could lead to a future with untreatable gonorrhea. Thus, the development of novel anti-N. gonorrhoeae drugs is urgently needed. N. gonorrhoeae FA1090 is the only strain reported to be used for in vivo mouse models because of its natural resistance to streptomycin. Streptomycin is a necessary antibiotic utilized in the mouse model to inhibit the commensal flora in the lower genital tract of mice to enhance N. gonorrhoeae colonization. However, this strain is susceptible to all antibiotics used to treat gonorrhea, and therefore, it is not suitable for drug discovery. To test the efficacy of new therapeutics against clinically important N. gonorrhoeae isolates, such as ceftriaxone-resistant and azithromycin-resistant strains in vivo, streptomycin resistance is a required phenotype for performing the in vivo mouse model. Thus, there is a requirement to develop N. gonorrhoeae strains that are simultaneously resistant to streptomycin as well as standard-of-care antibiotics, azithromycin and ceftriaxone. In this study, using allelic-exchange procedures, we constructed a N. gonorrhoeae mutant that is resistant to both streptomycin and azithromycin, and another N. gonorrhoeae mutant that is resistant to both streptomycin and ceftriaxone. The minimum inhibitory concentrations of standard antibiotics were determined against the newly constructed strains compared to their wild-type strains. When used in N. gonorrhoeae genital tract infection mouse model, mice were colonized with the new mutants for 14 days like N. gonorrhoeae FA1090. Overall, our results indicate that the newly constructed mutants proved to be suitable to be utilized in the N. gonorrhoeae infection mouse model for drug discovery studies.

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Hope on the horizon: HIV protease inhibitor, atazanavir overcomes azole resistance in Candida auris infection

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Candida auris represents an urgent public health threat that has been linked to numerous outbreaks around the world and is associated with a significantly high mortality rate. Therapeutic options are currently limited to 3 main classes of antifungals (azoles, polyenes, and echinocandins) to treat C. auris infections. The limited treatment options and the upsurge of drug resistance in C. auris, prompted us to evaluate a library of FDA-approved drugs for their ability to restore the anti-Candida activity of azole antifungal agents. We identified the HIV protease inhibitor atazanavir, as a co-drug that can overcome azole resistance in C. auris. Atazanavir displayed a remarkable in vitro synergistic activity with itraconazole against 19/19 C. auris isolates with a fractional inhibitory concentration index (ΣFICI) that ranged from 0.09 to 0.38. Moreover, atazanavir restored the fungistatic activity of itraconazole against C. auris in an in vitro time-kill assay. Mechanistic studies revealed that atazanavir significantly interfered with C. auris efflux pumps which resulted in an increase in the Nile red fluorescence by ~50%. Additionally, atazanavir inhibited glucose transport and ATP synthesis, which caused the glucose utilization and ATP content in C. auris to decrease by 30% and 20%, respectively. When evaluated in a mouse model of disseminated candidiasis, the combination of atazanavir/itraconazole, along with ritonavir that serves as a bioavailability booster, significantly reduced C. auris' burden in murine kidneys, generating a 1.15-log10 colony forming unit (CFU) (~93%) reduction. Altogether, the data indicate that atazanavir is a potent azole chemo-sensitizing agent that merits further investigation.

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Discovering Antiviral Treatments for Alphaviruses Through Proteomic Analysis of the VEEV E1 Glycoprotein

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Venezuelan Equine Encephalitis virus (VEEV) is an alphavirus that can cause febrile disease, encephalitis and sometimes death in humans and equines. This emerging infectious disease currently has no FDA approved treatments or vaccines. Host protein interactions with the E1 fusion glycoprotein of VEEV remain mostly unknown, presenting an untapped source for potential antiviral targets. As a means to effectively isolate the E1 protein and characterize host interactors, we designed a VEEV TC83 molecular clone with a tag inserted in the C-terminal of the E1 protein. Utilizing this tagged virus (VEEV TC83 E1-V5) and mass spectrometry, we have discovered 182 host interactors of E1 after normalization. Many of these proteins are involved in pathways known to be modulated during viral infection, including the unfolded protein response and autophagy. E1 interaction with protein disulfide isomerase family a member 6 (PDIa6), valosin containing protein (VCP) and ER lipid raft associated 2 (ERLIN2) was further verified through co-immunoprecipitation. Inhibition of PDIa6 with the PDI inhibitor Loc14 resulted in decreased viral titers and viral RNA levels. These data suggest that E1 interacts with VCP, PDIa6 and ERLIN2 to facilitate viral replication. Mechanism of action studies are ongoing to further elucidate the role of these host interactors in the VEEV replication cycle.

Support: DTRA

P39
Single Dose Pharmacokinetics of Pimobendan in Healthy Adult Horses

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Background: Pimobendan is an inodilator approved for treatment of canine cardiac disease. The pharmacokinetics have not been investigated in horses.
Hypothesis/Objectives: This study evaluated the pharmacokinetics of oral Vetmedin (V) in horses. Additional objectives were to determine the bioequivalence of compounded pimobendan capsules (C) and suspension (S) and the effects of sample site on plasma drug concentrations. Animals: Six privately-owned healthy horses (5 mares, 1 gelding). Methods: All horses received a single 0.5mg/kg dose of pimobendan via oral syringe. The initial two horses received C, S, or V using a crossover design with a minimum 1-week washout period. Samples were collected simultaneously from lateral thoracic and jugular catheters before and after drug administration at predetermined times. Differences between formulation and sample site were analyzed by one-way ANOVA. Four horses received (V) only with jugular samples collected. Analysis was by LC-MS/MS and noncompartmental pharmacokinetics for (V). Results: No significant differences were noted between formulations or sample site ($P > 0.05$). Concentrations in compounded formulations were 88%(S) and 90%(C) of label. For V, mean (±SD) maximum plasma concentration (Cmax) was 4.96 ± 2.13ng/mL at 2.17 ± 0.98hr, and area under the curve ($AUC_{0-\infty}$) was 22.1 ± 8.8hr*ng/mL. Concentration of the active metabolite (O-desmethylpimobendan) was below the limit of detection (0.07ng/mL) for all samples. Conclusions and clinical importance: At 0.5mg/kg PO, pimobendan plasma concentrations were considerably lower than reported in dogs. There was no evidence of oral transmucosal absorption. Pimobendan is poorly absorbed in horses, regardless of formulation, and appears unlikely to have clinical effects.

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P40

Pre- and Post-Phacoemulsification Morphologic Iridocorneal Angle Analysis by Anterior Segment Gonioscopic Imaging as a Predictor of Glaucoma in Dogs

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Purpose. To serially assess iridocorneal angle morphology following phacoemulsification in dogs to determine risk of post-operative glaucoma development. Methods. Client-owned dogs presenting for surgical removal of cataract by phacoemulsification were included. Each eye underwent serial anterior segment imaging using a retinal imaging camera (RetCamTM). In addition to standard-of-care post-phacoemulsification management, images of the complete iridocorneal angle were obtained in four quadrants both pre-operatively and at follow-up up to one year post-operatively. Each image was evaluated to measure both angle width and pectinate ligament dysplasia (PLD) severity. Together, angle width and PLD are interpreted per a previously described equation (Zibura 2020) to determine an overall ZibWest Angle Index score for each eye at each timepoint. Results. 40 patients have been enrolled, and five patients (10 eyes) have been measured through the 6-9 month timepoint and statistically evaluated, all of which have not been diagnosed with postoperative glaucoma. A statistically significant difference in ZibWest Angle Index was observed between the following: pre-operative & 3-4 months post-operative (p=0.0027); pre-operative & 6-9 months post-operative (p=0.0009); 1-2 weeks post-operative & 3-4 months post-operative (p=0.0328); 1-2 weeks post-operative & 6-9 months post-operative (p=0.0117); 3-6 weeks & 6-9 months post-operative (p=0.0453). Though not always statistically significant, a decrease in ZibWest Angle Index was observed at each subsequent timepoint. Conclusion. Serial imaging of the iridocorneal angle post-phacoemulsification reveals a decrease in ZibWest score even in non-glaucomatous eyes. Supported by the Virginia-Maryland College of Veterinary Medicine Veterinary Memorial Fund. None.

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Functional Characterization of TgTKL1 Kinase in Toxoplasma gondii Pathogenesis

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Toxoplasma gondii is an important human pathogen that causes miscarriage in pregnant women, blindness and cognitive impairment in newborn children. Available treatments are compromised by toxic side effects, inability to treat the chronic form of the disease, which urges the need to develop new therapeutic strategies. Therefore, identifying parasite's factors involved in pathogenesis could lead to finding new drug targets. Tyrosine Kinase-Like (TKL) family kinases are predicted to play critical roles in Toxoplasma's growth, and they are poorly studied. We focused on TgTKL1, and our studies showed that this kinase is important for parasite growth in vitro and virulence in vivo. TgTKL1 contains four different domains: RNI, Enhanced Disease Resistance 1 (EDR1) domain, kinase domain and Nuclear Localization Signal (NLS) motif and contributions of these domains to TgTKL1's functioning remains unknown. To define the role of these domains in TgTKL1's function, we generated different mutant strains using CRISPR-Cas9. To determine significance of kinase domain, we successfully generated a kinase mutant strain and interestingly, it displayed defects in growth and virulence like the null mutant. RNA seq analysis showed that invasion related genes are downregulated in the kinase mutant including TgSUB1 (subtilisin 1), a protease required for microneme proteins processing during host-cell invasion. Accordingly, TgTKL1 kinase mutant displayed impaired processing of micronemal proteins revealing that kinase activity is crucial for TgTKL1 function. Furthermore, we generated mutants in the NLS motif to determine if localization to the nucleus is critical for TgTKL1 function. Additionally, we are also in the process of generating RNI and EDR1 deletion mutants and once these strains are generated, they will be subjected to different phenotypic assays including growth, invasion, microneme secretion and virulence. Moreover, multiple approaches including quantitative phosphoproteomics, immunoprecipitation and proximity ligation assays will be used to understand the signaling pathways mediated by TgTKL1.

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Evaluation of a feline optimized TSH assay in cats with hyperthyroidism and with non-thyroidal illness

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About 10% of hyperthyroid cats have a normal total T4 requiring further testing to make the diagnosis. In people, thyroid stimulating hormone (TSH) can be used to make the diagnosis. As a specific feline assay is not currently available, TSH is measured using the canine assay (cTSH). However, this assay cannot differentiate between subnormal and low-normal TSH concentrations in cats, making it a poor test for diagnosing feline hyperthyroidism. A novel feline optimized TSH assay (fTSH, Truforma by Zomedica) was recently developed. It differentiates better between euthyroid and hyperthyroid cats compared to cTSH. However, the effect of non-thyroidal illness (NTI) on fTSH has not been evaluated. Objectives: Comparison of fTSH and cTSH concentrations among hyperthyroid cats, cats with non-thyroidal illness (NTI), and healthy cats. Evaluation of sensitivity and specificity of fTSH to diagnose hyperthyroidism. Hypotheses: Hyperthyroid cats have lower fTSH compared to healthy and ill cats. The fTSH detects more hyperthyroid cats than cTSH. Cats with NTI have similar fTSH and cTSH concentrations as compared to healthy cats. Prospective study consisting of hyperthyroid cats, healthy cat, and cats with NTI. Serum was collected to measure T4 and cTSH (Immulite 2000) and fTSH (Truforma platform) concentrations in all cats. Hyperthyroid cats had a thyroid scintigraphy performed to confirm hyperthyroidism. Healthy and NTI cats had T4 repeated 3 months from enrollment if available for follow up to rule out subclinical hyperthyroidism. The fTSH was compared among groups using Kruskal Wallis followed by Wilcoxon pairwise method. Significance was set at $P < 0.05$.

Preliminary results of 29 hyperthyroid cats, 29 healthy cats and 30 cats with NTI. The mean age of healthy, hyperthyroid, and NTI cats were 8.7 yrs, 11.6 yrs, and 12.1 yrs, respectively, with healthy cats being significantly lower in age compared to the other groups. There is no difference between mean fTSH and cTSH concentrations between healthy and NTI cats. TSH concentrations from both assays are significantly lower in hyperthyroid cats than in healthy and NTI cats ($P<0.001$). Five (17%) hyperthyroid cats had a normal fTSH but undetectable cTSH. One cat has a normal cTSH (0.035), but undetectable fTSH (<0.008). Conclusions: The fTSH identifies normal TSH in healthy cats more often than cTSH and appears to not be affected by NTI. The fTSH can be a useful tool for the diagnosis of feline hyperthyroidism.

Support: Zomedica

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Characterization of transcription factors in acute toxoplamosis

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Toxoplasma gondii is an obligatory intracellular protozoan parasite that is extremely successful in chronically affecting one-quarter of humans around the globe. In humans, Toxoplasma divides by a process known as endodyogeny, and the egress of newly formed daughter parasites results in host cell destruction. The lytic mode of propagation is responsible for pathology during toxoplasmosis and identification of unique parasite factors involved in different stages including invasion, replication, and egress is important for developing new therapeutics. ApiAP2s are a family of transcription factors in Toxoplasma that are quite divergent from the transcription factors seen in higher eukaryotes and hence are considered good drug targets. We are specifically focussing on two unexplored transcription factors, AP2 X-7, and AP2 XI-2, that has been predicted to be essential for Toxoplasma propagation. To determine the function of these genes we took a conditional knockdown approach using a recently developed AID (Auxin Inducible Degron) system. Accordingly, we successfully inserted the mAID HA epitope tag at the C-terminus of these genes using CRISPR-Cas9 technology. Immunofluorescence analysis (IFA) revealed that both proteins localize to the parasite nucleus. Our future goals are directed towards validating the conditional knockouts and then performing phenotypic analysis on these mutants that include determining changes in gene expression, identifying the interactome, and also dissecting the signaling pathways regulating the function of these transcription factors.

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Combined live oral priming and intramuscular boosting regimen with Rotarix and a nanoparticle-based multivalent rotavirus vaccine confers significant protection against both G4P[6] and G1P[8] human rotavirus infection in gnotobiotic pigs

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Human rotavirus (HRV) is the causative agent of severe dehydrating diarrhea in children under the age of five, resulting in up to 215,000 deaths yearly. With the introduction of live, oral attenuated vaccines nearly two decades ago, these deaths now almost exclusively occur in low and middle-income countries where vaccine efficacy is the lowest. Reasons for decreased efficacy in these areas include gut dysbiosis, concurrent use of other live oral vaccines, enteric viral infection, and malnutrition. These factors make parenteral vaccines for HRV particularly attractive, as they avoid many of the issues associated with oral vaccines. Our collaborators at Cincinnati Children's Hospital Medical Center developed a nanoparticle-based nonreplicating, parenteral HRV vaccine, utilizing the shell (S) domain of the capsid of norovirus (NoV). The S domain of NoV self-assembles into a nanoparticle with exposed C-termini, which are amenable to the insertion of foreign proteins. In this vaccine, HRV's VP8* from the three most predominant P types were fused to the exposed C-termini, forming the S60-VP8* nanoparticle cocktail vaccine. The safety, immunogenicity and protective efficacy of this cocktail vaccine as a two-dose intramuscularly administrated regimen, as well as a booster using one dose of the commercial oral Rotarix vaccine as a priming immunization was evaluated in the gnotobiotic pig models of P[6] and P[8] HRV infection and diarrhea. The prime/boost regimen provided partial protection from diarrhea and significantly delayed the onset of virus shedding in pigs challenged orally with the virulent Wa (G1P[8]) HRV. This regimen also significantly shortened mean duration, mean peak titer and AUC of virus shedding after challenge with Arg (G4P[6]) HRV. Both vaccine regimens were highly immunogenic and induced significantly higher titers of HRV-specific serum IgG, IgA and virus neutralizing antibodies in both challenge groups. Prime/boost-vaccinated pigs challenged with Wa HRV had significantly higher P[8]-specific IgG antibody secreting cells (ASCs) in the spleen post-challenge. Prime/boost-vaccinated pigs challenged with P[6] HRV had significantly higher numbers of P[8]-specific IgA ASCs in the spleen, as well as significantly higher numbers of P[6] and P[8]-specific IgG ASCs in the ileum post-challenge. These results suggest the promise and warrant further investigation into the oral priming and parenteral boosting strategy for future HRV vaccines.

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P45

Neutrophils in the Tumor Microenvironment

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This literature review aims not only to answer how neutrophils affect tumor growth and survival; but serves to examine the interaction of neutrophils in the tumor microenvironment, as well as to elucidate potential therapeutic applications for neutrophils in cancer immunotherapy. A PubMed search was performed using the terms “cancer”, “neutrophils”, and “progression”. The research was conducted based off the findings from a total of 8 studies. Analysis of the studies demon started that neutrophils play an important dual mechanistic role in tumor promotion and inhibition. The function and plasticity of tumor associated neutrophils is affected by the surrounding tumor microenvironment, leading to two polarized states (N1 and N2). These two dissonant states are responsible for a wide variety of downstream effects culminating in either tumor progression or suppression. Clinical and therapeutic potential centers around the polarization states of the tumor associated neutrophils (TANs), neutrophil elastase (ELANE enzyme) therapy, metabolic programming to stimulate adaption in target organs, the inhibition of myeloid checkpoints, as well as immunoediting of neutrophils.

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Exploiting virus-host interactions to develop novel inhibitors against Venezuelan equine encephalitis virus

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Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne, positive sense, single-stranded RNA virus that belongs to the genus Alphavirus. VEEV can infect both equines and humans, with associated neurological complications in ~14% of human cases. Due to its low infectious dose, ease of aerosolization and manipulation, this virus is classified as a select agent by both the CDC and USDA. However, there are currently no FDA-approved therapeutics or licensed vaccines against VEEV infection in humans. The VEEV capsid protein is an essential virulence factor of VEEV. The capsid protein can simultaneously bind to the host's nuclear import receptors, importin α/β1, and the host export receptor, CRM1 to form a tetrameric complex. This complex accumulates at the nuclear pore channel, halting nucleocytoplasmic trafficking, downregulating host transcription and inhibiting cellular antiviral response. Moreover, VEEV TC83 Cm, with a mutated non-functional nuclear localization sequence within the capsid, failed to downregulate cellular transcription and antiviral response. This suggests that the nuclear import of VEEV capsid is pertinent for pathogenesis and could be exploited as an attractive target for therapeutic development. We hypothesized that chemical inhibitors capable of disrupting the interaction of capsid with importin α/β1 should increase cellular antiviral response, resulting in reduced viral titers and rescue of cells from VEEV-induced cell death. Two small molecule inhibitors, I2 and 1564, were designed to disrupt the interaction between capsid and importin α. These inhibitors were well tolerated by HMC3 microglia cells with CC50 of >250 μM and >500 μM for I2 and 1564, respectively. These compounds impacted VEEV TC83 titer with >1 log10 decrease at 9 hpi. Furthermore, I2 displayed an EC50 of 2.96 μM and 1564 an EC50 of 5.38 μM against VEEV. Both compounds also rescued infected cells from VEEV-induced cell death. In order to evaluate the impact of these compounds on the capsid-importin α interaction, we cloned two viruses that contain a V5 tag at the N-terminus of the capsid, TC83 V5-C and TC83 V5-Cm. The replication kinetics of these new viruses were similar to that of parental TC83 and TC83 Cm. Future studies will involve evaluating the impact of these compounds on capsid-importin interaction and capsid localization using co-immunoprecipitation and confocal microscopy.

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Zika virus recruits an importin to its replication site as a proviral factor

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Zika virus (ZIKV) is an arbovirus, which is an important human pathogen. ZIKV is a positive-sense, single-stranded RNA virus, which replicates in the cytoplasm. We discovered that Karyopherin α6 (KPNA6), a transport factor in the nucleocytoplasmic trafficking system, is needed for ZIKV replication. First, we found that ZIKV infection induced elevation of KPNA6 protein level compared to mock-infected cells. Furthermore, knockout of KPNA6 in Vero cells inhibits ZIKV replication, while exogenous expression of full-length KPNA6 in KPNA6-deleted cells rescues ZIKV replication. However, a truncated KPNA6 fails to do so. The effect of KPNA6 on ZIKV replication indicates that KPNA6 is a proviral factor. Further study shows that it has no effect on ZIKV attachment and internalization. We also found KPNA6 co-localizes with ZIKV double-stranded RNA (dsRNA), an indispensable replication intermediate, while the majority of KPNA6 of mock-infected cells is present in the nucleus. The ZIKV-induced KPNA6 relocation led us to posit that ZIKV recruits KPNA6 to the replication site to facilitate viral proliferation. To determine if KPNA6 is present in the ZIKV replication complexes, we first verified that these membranous structures are detergent-resistant and then isolated ZIKV RCs through flotation in a sucrose density gradient centrifugation. Indeed, KPNA6, ZIKV NS2B, NS3, and NS5 proteins were detected on the top fraction. Also, most negative-sense ZIKV RNA that are assumed to be present in the replication complexes was detected in the top fraction, which suggests that ZIKV replication complexes are present in this fraction. Together, our data indicate that ZIKV recruits KPNA6 to the replication site to assist viral proliferation.

P48

Optimizing centrifugation parameters for cooled canine semen processing

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Semen processing is critical during preparation for cooled shipments or sperm cryopreservation in dogs. The process involves centrifugation and re-suspension of the sperm pellet to appropriate volumes using semen extender. Our hypothesis was that longer centrifugation times at higher speed will lead to increased sperm recovery rates (RR) but reduced sperm viability, motility, and percent normal morphology. Semen was collected from 14 healthy client-owned stud dogs of medium to large breeds and mean age of 3.3 years. Inclusion criteria were negative Brucella canis serology, ejaculate with ≥70x106/ml sperm concentration, ≥70% total motility and ≥40% normal morphology. Ejaculates were divided into six equal volume aliquots, subjected to one of six centrifugation treatments (400g, 720g, and 900g, each for 5 or 10 minutes; denoted as A-F, respectively). Sperm pellet was re-extended to original aliquot volume with CaniPlus Chill LT (Minitube) and cooled at 4°C for 48 hrs. Sperm viability was evaluated by mixed model two-way ANOVA using percentage change from baseline, and sperm RR, subjective total motility (TM) and computer-assisted sperm analysis (CASA) total (TM) and progressive motility (PM)), % normal morphology were analyzed by a linear generalized estimating equations model using change from baseline, comparing treatment groups and time points (T0: initial raw as baseline, T1: post-centrifugation, T2: 24 hrs, and T3: 48 hrs). $P<0.05$ was considered significant.

Sperm RR was similar among the six treatment groups ($p\geq0.062$; 98.8-102.1%). The viability significantly declined at T2 and T3 compared to T1 ($p\leq0.001$) in all treatment groups. Subjective TM decreased over time in all groups (T1 to T2 $p\leq0.041$, T1 to T3 $p\leq0.0002$, T2 to T3 $p\leq0.016$). CASA TM showed significant decline by T3 (T1 to T3 $p\leq0.023$, T2 to T3 $p\leq0.031$) for all groups. CASA PM decreased from T1 to T3 ($p\leq0.004$) for all groups; A, C, D, E, and F from T2 to T3 ($p\leq0.013$) and T1 to T2 for C and E ($p\leq0.044$). Percent normal spermatozoa decreased already by T2, independent of treatment groups (T1 to T2 $p\leq0.0001$, T1 to T3 $p\leq0.0001$). For healthy, fertile stud dogs, semen parameters after centrifugation and standard shipping conditions were not significantly affected by centrifugation speed or time, but all treatment groups showed declines over the 48 hrs period of cooled storage. The centrifugation speeds of 400-900g for 5-10 min duration are appropriate for canine semen processing.

Support: Dr. JoAnne S O'Brien Endowment Fund at Virginia Tech

P50

Development of a novel approach to broadly-protective anti-enterovirus vaccines

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Enteroviruses are arguably the most numerous human viral pathogens. Due to high antigenic diversity, the development of traditional capsid-targeting vaccines is feasible for only few enteroviruses. Yet, their replication proteins are much more conserved and are essentially interchangeable, as evidenced by the extensive recombination of enterovirus genomes. We observed that upon immunization with live polioviruses of mice and non-human primates, the animals develop antibodies not only against the capsid proteins but also against a number of non-structural proteins, indicating that antigens from conserved non-structural proteins are presented to and recognized by the immune system. We set to investigate if a vaccine based on expression of conserved replication proteins only could be protective, and if that protection could be extended to antigenically diverse enteroviruses. We trans-packaged the poliovirus replicon RNA coding for P2P3 proteins using our previously developed Newcastle Disease Virus (NDV) vectored vaccine expressing poliovirus virus-like particles. This trans-packaging system based on efficiently replicating poliovirus P2P3 RNA and NDV allows cost-effective production and purification of packaged replicons. These replicons can be administered similarly to live poliovirus vaccine, but the capsid proteins are present only in the original inoculum and are not produced upon replicon RNA replication. Preliminary experiments in transgenic mice demonstrate that replicon immunization indeed promote a much higher presentation of non-structural proteins to the immune system, however a significant development of antibodies against capsid proteins was also observed, indicating that an alternative replicon RNA delivery system is required to completely remove the input of immunodominant capsid proteins.

P51

EGR1 mediates distinct programs of gene expression regulation in excitatory and inhibitory neurons

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Brain development and neuronal cell specification are accompanied with epigenetic changes to achieve diverse gene expression regulation. Interacting with cell-type specific epigenetic marks, transcription factors bind to different sets of cis-regulatory elements in different cells. Currently, it remains unclear how cell-type specific gene regulation is achieved for neurons. In this study, we generated epigenetic maps to perform comparative histone modification analysis between excitatory and inhibitory neurons. We found that neuronal cell-type specific histone modifications are enriched in enhancer regions containing abundant EGR1 motifs. Further CUT&RUN data validated that more EGR1 binding sites can be detected in excitatory neurons and primarily located in enhancers. Integrative analysis revealed that EGR1 binding is strongly correlated with various epigenetic markers for open chromatin regions and associated with distinct gene pathways with neuronal subtype-specific functions. In inhibitory neurons, the majority of genomic regions hosting EGR1 binding sites become accessible at early embryonic stages. In contrast, the enhancers in excitatory neurons hosting EGR1 binding sites gained their accessibility during postnatal stages. Altogether, this study underscores the importance of neuronal activity induced transcription factors to the establishment of cell-type specific gene regulation in neurons.

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P52

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Streptococcus agalactiae and Staphylococcus aureus are the leading pathogens responsible for bovine mastitis and an array of human diseases. The global burden is only aggravated by the ascend of drug resistant strains such as methicillin resistant Staphylococcus aureus (MRSA). An alternative to antibiotic resistance comes from bacteriophages, the viruses of the bacteria. Some bacteriophage cell wall hydrolases, termed endolysins, responsible for lysis the bacterial cell from within during the phage life cycle, also demonstrate the ability to break down the peptidoglycan of a bacterial cell wall when applied externally. This peculiar characteristic of endolysins along with their immunity to efflux pumps, penicillin binding proteins, alterations of metabolic pathways, or other mechanisms of traditional antibiotic resistance make them excellent candidates to combat these drug resistant bacteria. A typical endolysin targeting gram-positive bacteria employ a modular structure, with an N-terminal enzymatically active domain (EAD) which cleaves the peptidoglycan, linked to a C-terminal cell wall binding domain (CBD) which binds to the surface proteins/carbohydrates in the cell wall. Harvesting the knowledge garnered over the years and employing the tools generated over a decades of research on these endolysins, the aim is to create a chimeric endolysin that combats both S. agalactiae and S. aureus including the drug resistant strains.

P53

Spatiotemporal multi-omics analysis of the hippocampal-amygdala circuit during contextual fear memory consolidation

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The consolidation of contextual fear memories requires the crosstalk among multiple brain regions, including the hippocampus and amygdala. Despite the advance in single cell transcriptomic techniques, tracing the dynamic gene expression across the hippocampal-amygdala circuit remain challenging. Here we integrated high resolution spatial transcriptomics with single nuclei multi-omics techniques to systematically investigate the spatiotemporally transcriptional programs across the hippocampal-amygdala circuit during the encoding and retrieval of contextual fear memory. It reveals highly dynamic gene expression programs in the subregions of hippocampus and amygdala. Our data provides new insight into the transcriptional regulation during memory consolidation.

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P54

Single Cell Multi-Omics Analyses Reveal Mouse Hippocampal Gene Expression Aberrations with Excess Maternal Folate Supplementation

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The supplementation of folic acid, a synthetic form of folate, during pregnancy is a preventative approach for neural tube defects in offspring. Despite the well-known benefits of folate supplementation, recent studies raised concerns about the adverse effects of excess maternal folic acid supplementation. With mouse models, high folate intake during pregnancy has been linked to delayed cerebral cortical neurogenesis and short-term memory impairment in offspring. In this study, we examined the influence of excess maternal folic acid supplementation on mouse hippocampal development. Via integrating single nuclei RNA-seq and ATAC-seq with high resolution spatial transcriptomics techniques, we aim to systematically investigate the influence of excess maternal folate intake on gene expression and chromatin accessibility in the hippocampus of the offspring. Single cell analysis revealed aberrant gene expression across multiple cell types. In summary, this study provides new insight into the effect of excess maternal folate supplementation on the brain development in the offspring.

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ABSTRACTS

Oral Talk 1 Session I

Outcomes of hyperthyroid cats treated with radioiodine (I-131) using a variable-dose protocol determined by serum thyroxine (T4) concentration

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Radioiodine (I-131) is considered the gold standard to treat hyperthyroidism in cats. The most successful I-131 dosing protocol investigated to date is based upon a combination of serum thyroid hormone concentrations and scintigraphy findings, however most clinicians lack easy access to scintigraphy. The primary objective of this retrospective study was to report outcomes of hyperthyroid cats treated with I-131 using a variable-dose protocol determined by serum thyroxine (T4) concentration. A secondary objective was to report the distribution of pre-I-131 characteristics in this population of cats (patient, scintigraphic, clinicopathologic factors). Hyperthyroid cats treated with I-131 using a T4-based variable-dose protocol that had ≥6-month follow-up data (serum T4/TSH concentrations) were included. 336 cats treated at two referral hospitals met inclusion criteria. Outcome categories, defined by ≥6-month post-I-131 serum T4/TSH concentrations, included the following: overtly hypothyroid (low T4, high TSH), subclinically hypothyroid (normal T4, high TSH), euthyroid (normal T4/TSH), and hyperthyroid (high T4, low TSH). Distribution of the following factors was determined: patient age, sex, breed, body weight; scintigraphic pattern & percent thyroidal uptake of 99mTc-pertechnatate (TcTU); serum T4/TSH concentrations. Of 336 cats included, 27.1% became euthyroid, 15.2% became overtly hypothyroid, 43.2% became subclinically hypothyroid, and 15.0% remained hyperthyroid. 44% of cats were male, 56% were female. Median age was 12 years (range 4-19). A majority (91.1%) were domestic short-, medium- or long-haired cats; the remainder (8.9%) were distributed amongst various other breeds (Maine Coone, Siamese, etc). Median body weight was 4.2 kg (range 1.8-12.6). Median TcTU was 3.5% (range 0.6-66.4). Scintigraphic pattern distribution: 48.5% unilateral, 25.3% bilateral-symmetric, 25.4% bilateral-asymmetric, 0.8% multifocal. Ectopic thyroidal tissue was detected in 1.1% of cats. Median pre-treatment serum T4 concentration was 101.7 nmol/L (range 12.9-499.4), and a vast majority of cats (97.8%) had a serum TSH concentration below the detectable range of the analyzer. Our dosing protocol based on serum T4 concentration resulted in a high rate of iatrogenic hypothyroidism. Alternative T4-based variable I-131 dose-protocols utilizing lower dosages should be investigated in the future to reduce the rate of iatrogenic hypothyroidism.

Support: Office of Research and Graduate Studies

ABSTRACTS

Oral Talk 2 Session I

Teaching an old drug a new trick: Repurposing the anti-inflammatory FDA-approved drug, auranofin, to treat Neisseria gonorrhoeae

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Neisseria gonorrhoeae, the second most common bacterial cause of sexually transmitted infections, is listed as an urgent-threat pathogen by the Centers for Disease Control and Prevention (CDC). Due to the growing prevalence of resistance development against the first-line treatment and several classes of antibiotics, the discovery of new anti-gonorrheal therapeutics is an urgent need. Drug repurposing significantly reduces the time and expense associated with traditional drug development. Herein, utilizing a drug repurposing approach, we screened 3,802 FDA-approved and clinical drugs against N. gonorrhoeae FA1090. A total of 14 novel non-antibiotic compounds were identified in the screening with significant anti-gonococcal activity. Auranofin, an FDA-approved anti-rheumatoid arthritis drug, was selected for further investigation due to its potent activity. A time-kill kinetics assay revealed that auranofin exhibited rapid bactericidal activity in vitro against N. gonorrhoeae, outperforming the drug of choice, azithromycin. Moreover, auranofin reduced the N. gonorrhoeae burden in a female murine model of vaginal infection by 91% and 96% after three and five days of treatment, respectively. In conclusion, our results indicate that auranofin merits further investigation for development as a future anti-gonorrheal therapeutic to replenish the dry pipeline of anti-gonorrhea medications

Oral Talk 3 Session I

The Role of Sympathetic Neuronal Pathways in Regulating HSV1 and HSV2 Genital Infection

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Herpes simplex virus (HSV) is one of the most common STIs and genital HSV lesions have been shown to increase three-fold the risk of acquisition and spread of other STIs such as HIV. This painful, life-long disease is estimated to affect over 85 million people in the US. HSV1 and HSV2 are closely related viruses with HSV1 typically associated with orofacial lesions and HSV2 associated with genital lesions, although these common morphologies are not exclusive of each other. HSV establishes life-long infection by traveling retrograde along neuronal axons after primary infection to establish latency in sensory and autonomic neuronal cell bodies that directly innervate the genitourinary system. The latent virus can then become reactivated by a variety of stimuli, traveling back down the neuronal axons to induce a recurrence of painful lesions. Within the autonomic nervous system, HSV1 shows a preference for the sympathetic pathways whereas HSV2 shows a preference for the parasympathetic pathways suggesting that autonomic pathways may contribute to the difference in recurrence frequencies of HSV1 and HSV2. To determine the contribution of the sympathetic nervous system to acute genital disease and recurrence frequency, guinea pigs were treated with 6-hydroxydopamine (6-OHDA) prior to infection to ablate sympathetic neuronal axons, making them unavailable for infection and the establishment of latency. Chemical ablation of sympathetic axons significantly reduced severity and neurological involvement during acute disease for HSV1, but not HSV2 ($p < 0.05$). Animals treated with 6-OHDA prior to infection also had reduced clinical recurrences by nearly 75% for HSV1 and by 50% for HSV2 ($p \leq 0.001$). Thus, sympathetic pathways play a significant role in acute disease severity and neurological involvement of HSV1, but not HSV2. Additionally, sympathetic pathways are responsible for a significant portion of HSV1 and HSV2 recurrences, with a greater impact on HSV1 than HSV2.

Support: NIH, VMCVM, Institute for Critical Technology and Applied Science

ABSTRACTS

Oral Talk 4 Session I

High frequency irreversible electroporation of glioma alters tumor-derived extracellular vesicles to mediate blood-brain barrier disruption

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Glioblastoma is the most common and deadly primary brain tumor. The efficacy of surgical resection, chemotherapy, and radiotherapy is limited due to the presence of infiltrative tumor cells that are shielded from effective delivery of systemic therapies by the blood-brain barrier (BBB). High-frequency irreversible electroporation (H-FIRE), a novel nonthermal tumor ablation therapeutic, applies pulsed electric fields to precisely ablate tumor tissue, while transiently disrupting the peritumoral BBB (pBBB). This can be exploited to enhance delivery of systemic therapeutics to the infiltrative cancer cells, but the mechanisms of H-FIRE-induced pBBB disruption are not fully defined. We hypothesize that bystander effects of H-FIRE tumor cell ablation disrupt the pBBB endothelium after H-FIRE ablation of glioma via the release of tumor-derived extracellular vesicles (TDEVs). TDEVs contain complex bioactive cargo and facilitate diverse communication between tumor cells and their microenvironments. We isolated and characterized TDEVs after H-FIRE treatment of F98 glioma cell suspensions after sham, sub-ablative, and ablative doses of H-FIRE, and found that fewer TDEVs were released after ablative doses. These fewer TDEVs released after ablative doses of H-FIRE increase permeability of a bEnd.3 cerebral endothelial cell Transwell® model of the BBB endothelium. To determine whether the cargo of the BBB-disrupting TDEV populations was distinct from that of the non-disruptive populations, we used mass spectrometry to determine relative differences in proteomic payloads of the TDEVs. TDEVs released by H-FIRE-ablated glioma cells, which disrupted the BBB endothelium in vitro despite their decreased abundance, had distinct proteomic cargo relative to the non-disruptive TDEVs released by sham-treated and sub-ablated glioma cells. Confocal microscopy demonstrated that TDEVs released by H-FIRE-ablated glioma cells are internalized by cerebral endothelial cells, while TDEVs released by sham- and sub-ablated glioma cells were not internalized. Taken together, this suggests that H-FIRE ablation of glioma cells alters proteomic cargo of TDEVs in a way that may contribute to disruption of the pBBB endothelium after H-FIRE ablation of glioma.

Support: NIH, Office of Research and Graduate Studies, Grayton Friedlander Memorial Fund, Center of Engineered Health

Oral Talk 5 Session I

Evaluation of neurofilament light chain as a biomarker in dogs with structural and idiopathic epilepsy

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Background: Neurofilament light chain (NfL) is a frequently used biomarker in human medicine for both diagnostic and therapeutic monitoring purposes in various neurologic diseases. It has yet to be evaluated in either humans or dogs with epilepsy. Hypothesis / Objectives: It was hypothesized that dogs with diagnosed structural epilepsy would have a significantly increased NfL concentration compared to dogs with idiopathic epilepsy. It was also hypothesized that idiopathic epileptic dogs with a recent onset of seizure activity would have a significantly increased NfL concentration compared to dogs with a chronically well controlled seizure frequency Animals: A total of 50 client owned dogs that presented to the Neurology service for an evaluation of seizures were enrolled in this study. There were 14 dogs with diagnosed structural epilepsy and 36 dogs with suspected idiopathic epilepsy. Methods: A total of 52 serum and 6 CSF samples were obtained for NfL concentration measurement using single molecule array technology (Simoa) for a prospective cohort study. Results: Serum NfL concentration was significantly increased in dogs with structural epilepsy when compared with dogs with idiopathic epilepsy ($p = 0.0009$). There was no significant difference in NfL concentration in dogs with an acute onset of seizures when compared to chronically well controlled epilepsy regardless of the underlying etiology. Conclusions / Clinical Importance: Neurofilament light chain may serve as a reliable biomarker for the differentiation of structural and idiopathic epilepsy.

Support: Clinical Applications Laboratory

Oral Talk 1 Session II

A novel 3D printed instrument enhances removal of equine guttural pouch chondroid prostheses

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Removal of guttural pouch (GP) chondroids in horses is essential to prevent transmission of Strangles but current chondroid removal techniques are time-consuming or have adverse effects. We aimed to determine if lavage and mechanical agitation using a 3D-printed instrument would be superior to lavage only, and to test 2 sites of instrument placement for chondroid removal via dorsal pharyngeal recess (DPR) fenestration. We hypothesized that the 3D instrument and placement via the fenestration would remove more chondroids, reduce procedure time and incur less soft tissue damage than lavage alone or instrument placement via the salpingopharyngeal ostium (SPO). Transendoscopic DPR fenestration was performed with Nd:YAG laser in cadaveric heads. Procedures to remove 50 plastic beads (12 mm diameter) from the GP were: 3DF: lavage via DPR with 3D instrument; LF: lavage only via DPR; 3DSPO: lavage via SPO with 3D instrument; LSPO: lavage only via SPO. Procedures were randomized in a cross-over fashion with 2 procedures performed per head for 10 repeats. 3DF and LF were randomized first then 3DSPO and LSPO. Damage was graded via endoscopy before and after fenestration and procedures. Heads were replaced between procedures if damage occurred. Lavage was 30 seconds on, 15 seconds off for 2-minute cycles at a maximum pressure of 200 mmHg and 2500mL/minute. Number of beads cleared, time for fenestration and to remove ≥96% of beads or have 3 consecutive cycles with no bead removal was recorded. Data were compared using a GEE model and Fisher's exact test ($p < 0.05$). Median time for fenestration was significantly slower ($p = 0.04$) for the first 13 heads (3DF, LF; 1225 seconds, range 563-2400) vs the next 10 heads (3DSPO, LSPO; 714 seconds, range 277-1294). Partial thickness, <5mm long mucosal damage occurred after 43.4% of fenestrations. 3D instrument procedures cleared a median of 48 beads, range 0-49, which was significantly higher than 6 beads, range 0-29 for lavage only procedures ($p < 0.001$) and had a faster median rate for bead removal of 24 beads/cycle, range 11.8-50, vs 0.66 beads/cycle, range 0-49, for lavage only procedures ($p < 0.001$). 3DF and 3DSPO had no difference in bead clearance ($p = 0.27$) or rate ($p = 0.45$). Post-procedure 30.4% of the heads had mucosal swelling with no difference between procedures ($p = 0.40$). The 3D instrument was efficient and safe to remove GP chondroids when used via a DPR fenestration or the SPO. Testing in live horses is needed.

Support: Virginia-Maryland College of Veterinary Medicine Equine Research Competition Grant (2021-2022)

ABSTRACTS

Oral Talk 2 Session II

A hepatitis B core antigen-based virus-like particle vaccine expressing SARS-CoV-2 T and B cell epitopes induces epitope-specific humoral and a cell-mediated immune responses

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The hepatitis B core antigen (HBcAg) contains three different sites that tolerate insertion of foreign immunogenic epitopes. After epitope insertion, the protein self-assembles into a virus-like particle (VLP) and can be used as a vaccine candidate. In this study, a HBcAg-based VLP expressing SARS-CoV-2 T-cell and B-cell epitopes was produced and tested for its immunogenicity and protection against SARS-CoV-2 in K18-hACE2 transgenic mice. The SARS-CoV-2 Spike protein epitopes used in this study were identified in silico and predicted to stimulate both humoral and cell-mediated immune responses. The recombinant HBcAg-SARS-CoV-2 protein was expressed in E. Coli cells and purified. A highly pure VLP product was confirmed by SDS-PAGE and VLP particle formation was confirmed by transmissible electron microscope. K18-hACE2 transgenic C57BL/6 mice were vaccinated intramuscularly and boosted twice with either vaccine or control VLP. Mice were challenged with SARS-CoV-2 virus three weeks following the final booster. Mice were monitored for up to eight days after challenge and evaluated for humoral and cell-mediated immune responses, clinical disease, and viral RNA load in the lung. A subset of mice was vaccinated but not challenged. This group was necropsied one week after the second booster dose to evaluate humoral and cell-mediated immune responses prior to virus challenge. The vaccinated mice in this subset showed a significant increase in epitope-specific IgG levels compared to baseline, had more memory CD8+ T-cells by flow cytometry, and higher IL-6 and MCP-1 expression by cytokine bead assay. While not statistically significant, there was also evidence of a Th1 response in 3/5 vaccinated mice, with high levels of IFN-γ and TNF production. Immunized mice had numerically lower viral RNA load in the lungs and slightly higher survival, but these differences are not statistically significant. These results indicate the HBcAg-based SARS-CoV-2 VLP vaccine can elicit both humoral and cell-mediated immune responses but was not protective against SARS-CoV-2 infection.

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ABSTRACTS

Oral Talk 3 Session II

Equine bone marrow-derived mesenchymal stromal cells disrupt the matrix of established orthopedic biofilms in vitro

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Biofilms protect bacteria from the host immune system and antibiotics, which enables bacteria to survive to cause chronic infection, morbidity and mortality in horses with orthopedic infections. Equine bone marrow-derived mesenchymal stromal cells (MSC) kill free-floating bacteria, but their ability to disrupt established biofilms is unknown. Our objective was to evaluate the ability of MSC to reduce established S. aureus or E. coli biofilms in vitro. We hypothesized that MSC would reduce the quantity of biofilm matrix, as determined by crystal violet staining and photography, and viable bacterial colony-forming units (CFU). We further hypothesized that MSC combined with an antibiotic, amikacin sulfate, would reduce these components to a greater extent than MSC alone, as has been demonstrated for free-floating bacterial reduction. MSC were cultured in antibiotic-free medium from cryopreserved bone marrow cells isolated from the sternum of 5 Thoroughbred horses aged 3-7 years. Biofilms of 2 x 105 CFU of S. aureus or E. coli were established for 24 hours in 24-well culture plates. Co-cultures were established by adding 1 x 106 passage 3 MSC in transwell inserts (0.4 µm, PET) to biofilms. Biofilms were treated for 24 or 48 hours in triplicate: 1) untreated (negative) control; 2) 500 µg/mL amikacin + 2% sodium dodecyl sulfate (positive control); 3) 30 µg/mL amikacin; 4) MSC; 5) MSC + 30 µg/mL amikacin; 6) medium only (contamination control). Biofilms were photographed after treatment, then biomass quantified via crystal violet stain and CFU quantified after proteinase K digestion. Data were compared using mixed model ANOVA with post-hoc Tukey comparisons ($p < 0.05$). Compared to untreated controls at both timepoints, biomass of S. aureus biofilms was reduced by MSC ($P < 0.001$) and MSC + amikacin ($P < 0.001$). Biomass of E. coli biofilms was reduced by MSC + amikacin at 24 ($P = 0.03$) and 48 hours ($P = 0.02$). Compared to untreated controls, MSC reduced CFU of S. aureus at 48 hours ($P = 0.04$) and MSC+ amikacin reduced CFU of S. aureus ($P < 0.001$) and E. coli ($P < 0.001$) at both timepoints. Image analysis revealed that MSC-treated biofilms were visibly smaller with less-defined central pellicles than untreated biofilms, suggesting disruption of the biofilm matrix. MSC effects depended on bacterial species. Evaluation of biofilm-MSC interactions, MSC dose and exposure time effects are warranted to evaluate MSC as a treatment for equine orthopedic infections.

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ABSTRACTS

Oral Talk 4 Session II

Human rotavirus replicates in the salivary glands and induces IgM responses in the facial lymphoid tissues of gnotobiotic pigs

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Human rotavirus (HRV) is the leading cause of severe diarrhea in children worldwide. The virus is typically associated with infection of the small intestine and transmission via the fecal-oral route. However, recent work has indicated that murine rotaviruses and noroviruses are capable of replicating in salivary glands and transmitting through saliva. In this study, we aimed to determine if HRV and rhesus rotavirus could replicate in the salivary glands of gnotobiotic (Gn) pigs. 20 neonatal Gn pigs were divided into 4 groups. At day 5 of age, pigs were orally inoculated with Rotarix, attenuated Wa strain HRV, recombinant rhesus rotavirus (rRRV), or diluent control. Rectal and nasal swabs were taken daily from post-inoculation day (PID) 0 until euthanasia to evaluate virus shedding. At PID 2, a subset of animals from each group was euthanized. Sections of the parotid, submandibular, and sublingual salivary glands and ileum were collected for virus detection. At PID 10, the remaining animals were euthanized. Tonsils, facial lymph nodes, and ileum were collected to enumerate numbers of HRV-specific IgM antibody-secreting cells. Intestinal contents and serum were collected to determine HRV-specific IgM titers. Presence of rotaviruses in fecal and nasal swab samples was detected using RT-qPCR in 50-88% of virus-inoculated pigs by PID 10. Rotavirus antigens were detected in the salivary glands and ileum of all virus-inoculated pigs, but not control pigs, at a similar intensity for both VP6 and NSP3 using immunofluorescence staining. IgM antibody-secreting cells in the ileum, tonsils, and facial lymph nodes, as well as high titers of IgM antibodies in serum and small intestinal contents were detected in all virus-inoculated pigs. These findings indicate that HRV and rRRV can replicate in salivary tissues and prime immune responses in the tonsil and facial lymphoid tissues of Gn pigs. These findings have important implications for HRV transmission and vaccine development.

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ABSTRACTS

Oral Talk 5 Session II

High Frequency Irreversible Electroporation (H-FIRE) of canine hepatocellular carcinoma results in immediate quantitative and qualitative changes in peripheral blood circulating extracellular vesicles

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High frequency irreversible electroporation (H-FIRE) is a non-thermal ablation technique using short, intense, bipolar electrical pulses to induce neoplastic cell death, currently trialed in the treatment of canine hepatocellular carcinoma (HCC). Ongoing in vitro work in our Lab suggests that H-FIRE may alter the release of extracellular vesicles (EV) with distinct effects on endothelial cells. Here, we hypothesize that H-FIRE treatment in dogs diagnosed with HCC results in changes in the peripheral blood circulating EVs. Dogs diagnosed with HCC were recruited for our clinical trials. H-FIRE was performed intraoperatively on the tumor, with subsequent surgical resection. Peripheral blood samples were obtained prior to and immediately after H-FIRE treatment. Plasma was isolated, aliquoted, and stored in -20°C. Plasma aliquots were filtered and ultracentrifuged to enrich for EVs and Nanoparticle Tracking Analysis was used to quantify EV concentration and size distribution. Transmission electron microscopy (TEM) was used to visualize the isolated EV's. Pairwise comparisons between pre and post treatment samples were conducted using commercially available statistical software. Five dogs diagnosed with HCC were treated with H-FIRE. The median circulating EV concentration post treatment significantly decreased (11x109 vs 94x109 particles/mL, p=0.0017). The mean EV diameter was significantly smaller post treatment (104.2 vs 91.2 nm, p=0.0020, and the mode was also significantly smaller post-treatment (90 vs 76.56 nm, p=0.0012). In addition, the mode size distribution indicates the presence of 2 distinct populations of EVs post-HFIRE. In vitro work in our Lab suggests that EVs released immediately after tumor cell treatment with lethal doses of H-FIRE could carry significant function in physiological and pathological processes. The work presented here suggests that H-FIRE treatment of canine HCC alters the population of peripheral blood circulating EVs, both in numbers and in their size. We plan to characterize the circulating EVs phenotypically and functionally from treated dogs.

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